Skin colour, colour preferences and retinal structure of pot bellied seahorse, *Hippocampus abdominalis*.

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at the University of Tasmania

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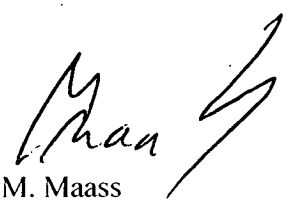
Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person; except where due reference is made in the text of this thesis.



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Abstract

This study investigated the effects of background colour (light and tank colour) on the skin colour changes, colour preferences and retinal structure in the pot – bellied seahorses *Hippocampus abdominalis*. Most of the seahorses changed their skin colour when held in different coloured tanks (red, yellow, green, blue and white) over 56 days. Exposure to coloured light (56 days), however did not have a significant influence on skin colour. Skin colour was measured every fortnight with the RAL design™ colour charts, where body parts (ventral, dorsal and spots) of fish were compared to the chart and their colour values recorded. In both experiments survival ($F = 0.68$, df 4, 19; $P = 0.617$; $F = 3.17$, df 4, 14; $P = 0.063$) and growth ($F = 0.66$, df 4, 19; $P = 0.353$; $F = 1.71$, df 4, 14; $P = 0.224$) was not significantly affected by any of the experimental colours. Measuring the colour preferences of non–adapted seahorses of different life stages in a free – colour – choice experiment for background colour or lighting colour (test colours: red, yellow, green, blue and white) resulted in a significant preference for a white background ($F = 39.89$ df 4, 45; $P < 0.001$) and green, blue and white light ($F = 1.82$, df 16, 225; $P < 0.05$). Seahorses adapted to different tank ($F = 9.01$ df 24, 100; $P < 0.001$) and lighting colours ($F = 14.37$ df 24, 100; $P < 0.001$) also preferred white in the background colour preference test, while in the light colour preference test, green was significantly preferred over the other test colours (background adapted fish: $F = 5.41$ df 24, 100; $P < 0.001$), (light adapted fish: $F = 9.61$ df 100, 125; $P < 0.001$). Histological examination of the retinal structure of the colour–adapted seahorses (56 d) showed that there was an influence of the green adaptation colour on retinal layer thickness and the blue adaptation colour on cone mosaics in both experiments (tank colour, light colour). Seahorses adapted to a green background or light had relatively thinner pigment epithelia and thicker absolute ganglion cell layers than seahorses of the other colours. Adaptation to a blue background or light caused the number of single cones to change in the retinal mosaic where the square units had two or three central single cones compared to other colours with just one central single cone.

Keywords: Skin colour, colour adaptation, colour preference, eyes, retinal structure, mosaics, *Hippocampus abdominalis*

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Chapter 1

General Introduction

1.1. Introduction

Fish in their natural habitat use colour to camouflage with their surroundings to avoid predation and to ambush prey, to display toxicity or danger and to attract mating partners. Colouration may be affected by many factors, including environment, nutrition and genotype. Adaptation to a change in environmental colour relies on fish perceiving their immediate environment and responding physiologically to it. However, information regarding the ability of fish to perceive colour is not understood in all species. The ability to perceive colour has very real and important repercussions for aquaculture and on the conservation of endangered fish species especially for restocking programs. Many fish which are hatchery–reared have behavioural deficits (Olla et al. 1994; Weber and Fausch 2003; Huntingford 2004; Salvanes and Braithwaite 2006). There can be abnormalities in predator avoidance and foraging due to abnormal response to visual cues or orientation. Fish released to the wild can have swimming deficiencies which often lead to higher mortalities than wild stock because they are not used to a natural environment (Olla et al. 1994; Oswald 2002; Weber and Fausch 2003; Huntingford 2004; Salvanes and Braithwaite 2006).

Studies focussed on understanding colour perception in fish and how this affects body colouration will enable a better understanding about environmental influences on fish behaviour. Information about colour perception and how it affects body colouration could also play an important role in producing specifically coloured ornamental fish, including seahorses, for the aquarium trade. Seahorses are produced in commercial aquariums for sale to reduce market reliance on wild populations for the ornamental trade as well as for the Traditional Chinese Medicine market (TCM). Since 2002 all Syngnathids were listed as endangered species in the Appendix II on the CITES list and companies started trying to farm seahorses to cope with the high demand. Research then was mainly focussed on general culturing and breeding techniques of seahorses (Bergert and Wainwright 1997; Woods 2000a; Woods 2000b; Woods 2001; Woods 2003a; b; c; Woods and Valentino 2003; Woods 2005a; Woods 2005b; Choo and Liew 2006). Now

companies are successful in farming certain species of seahorses like *Hippocampus abdominalis*, *H. erectus*, *H. capensis*, *H. barbouri*, *H. reidi*, *H. ingens*, *H. whitei* and *H. kuda*, the focus is on getting more valuable fish. Some of the mentioned species can be very drab like *H. abdominalis*, *H. whitei* and *H. kuda* and subsequently are of lower market value for ornamentalists. Therefore, there is considerable commercial interest in understanding to what extent holding or breeding conditions may change or influence the body colouration of easily cultured seahorses. Manipulating the environment to change the body colouration may also have influences on the fishes' growth performance due to their natural colour preferences, and physiological changes that may occur due to changing conditions e.g. retinal structure and behaviour (Kawamoto and Takeda 1951; Loukashkin and Grant 1959; Nagaishi et al. 1989; Fanta 1995; Boeuf and Le Bail 1999; Downing and Litvak 1999; Papoutsoglou et al. 2000; Rotllant et al. 2003; Carvalho et al. 2004; Volpato et al. 2004; Cobcroft unpublished).

Colour preferences of fish can give an indication of which environmental conditions best suit the specific species or if the species is capable of differentiating colours which most resemble their natural environment. Using preferred tank colours could enable farmers to create more natural rearing conditions to prevent stress, mortalities, aggression and behavioural abnormalities. Maybe it could also prevent fading of the skin colour which is a common problem in Aquaculture and causes high losses in market value of species like flounder, halibut, snapper, seabream and coral trout (Lin et al. 1998; Booth et al. 2004; Yamanome et al. 2004; Pavlidis et al. 2006).

To be able to determine colour perception the fishes vision, retinal structures and retinal pigments have to be examined and understood. This may also lead to an understanding of the fishes colour preferences during development and even to their natural behaviour during their life cycle. Much research has been done on eye development and retinal structure and pigmentation of larval fish but few studies have related the visual capability to skin colour display (Kurz 1920; Fricke 1973; Douglas and Lanzing 1980; Marshall et al. 2003a; Marshall et al. 2003b).

1.1.1. Vision of fish

The eye structure of fishes is very similar to those of other vertebrates (Fernald 1993). The fish eye is normally near-focused, and more distant objects can be focused by contracting the lens muscle (Fig. 1.1.). All other structural elements and retinal layers are the same as in other vertebrates. Colour vision is made possible by the cone cells and dim light vision by the rods, which lay in the back of the retina (Suworow 1959). Colour is the sensation that results from the stimulation of cone cells in the retina by light of certain wavelengths. Many fish have colour vision with a visible spectrum ranging from ultraviolet to red light (Nicol 1989). The spectral sensitivity of each species is dependent on their specific survival strategy (Fernald 1993; Fujii 1993).

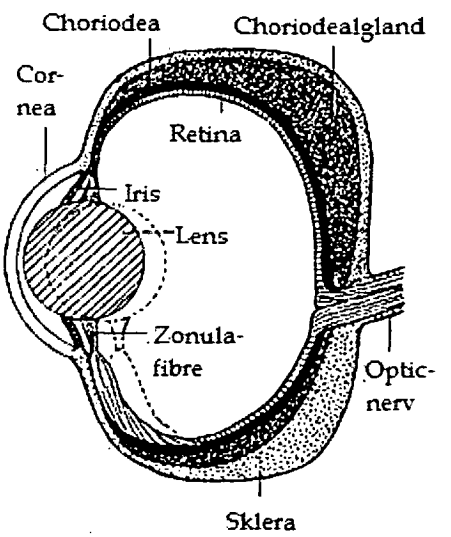

Figure 1.1: Cross – section of a fish eye with all major structures demonstrating lens movement (dashed line) (Spektrum 2006).

1.1.2. The pot bellied seahorse (*Hippocampus abdominalis*) as the examined species

Hippocampus abdominalis is a very common species in temperate Australian and New Zealand coastal and estuarine waters. Seahorses are teleosts belonging to the Syngnathidae. Seahorses are found in groups among macroalgae, seagrasses and rocky

reefs to a maximum depth of 50 m. *H. abdominalis* is sexually polygamous and breeds all year round with a peak in spring to summer. Seahorses reach a maximum length of 32 cm with a minimum length at sexual maturity in males of 8.7 cm and a gestation duration averaging 30 days. The egg diameter averages 1.8 mm and the hatchlings emerge at about 16 mm. Broods average about 300 newborn seahorses with a maximum reported brood size of 1116 (Lourie et al. 2004). Their natural colour patterns vary from pale, near-white to mottled yellow to variable brown with dark spots and blotches on head and trunk (Fig. 1.1). Their tail alternates with dark and light bands and the dorsal fin is mottled. Males have more dark blotches than females and commonly have a yellow dash near the top of the pouch (Lourie et al. 2004).

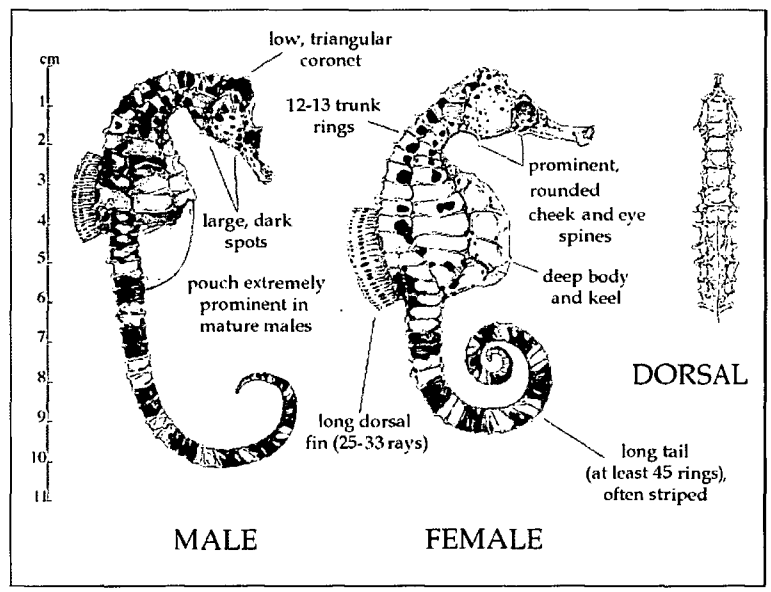

Figure 1.2: Illustration of a male and female seahorse, *H. abdominalis* (Lourie et al. 2004).

The main factor restricting expansion of ornamental seahorse culture is the darker body colouration of cultured seahorses which results in a low market value. The market seeks a golden – yellow body colouration (Wardley 2001) and although yellow fish are found in the wild, it is not understood what triggers the yellow pigmentation. If culture conditions could be altered to produce yellow fish, reliance on wild fish would decrease and the seahorse aquaculture could improve sales and prices. Various factors that may influence the colouration of *H. abdominalis*, including environmental conditions like

different background and lighting colours, testing fish for their colour preferences before and after exposure and their colour perception by histological eye preparations will be examined in this study.

1.1.3. Aims of the study

There is very little published data describing skin colour changes in the pot bellied seahorses (Wardley 2001). This study investigated the ability of *H. abdominalis* to change skin colour when influenced by two different environmental conditions. Therefore two systems were used, one with different coloured tanks and the other containing different coloured lights (Chapter 2).

The study also investigated colour preferences of *H. abdominalis* of different life stages both before and after the exposure to the new environmental conditions as mentioned above (Chapter 3).

The third aim of the study was to examine the eye of H. *abdominalis* through histological preparation and if changes in the environment have an influence on the retinal structure (Chapter 4).

Specifically the study addresses the following research questions:

- Does background colour influence the skin colour?
- Does lighting colour influence the skin colour?
- To what extend can both background and light change skin colour?
- Which skin colours can be produced?

- Which colours do pot bellied seahorses prefer?
- Does it change during their development?
- Does extended exposure to coloured background and light influence their colour preferences?
- To which extent do their preferences change?
- What does the retinal structure of *H. abdominalis* look like?
- Does adaptation to background or lighting colours induce any changes in retinal structure?

Chapter 2

Skin colour changes of the pot bellied seahorses *Hippocampus abdominalis* relative to background and light colour

2.1. Introduction

Skin colour changes are important for the crypsis or camouflage of animals. By blending in with their environment they avoid predation and capture prey (Waring 1963). Animals have developed the capability to change colour in response to temperature, mood, stress levels and social cues, rather than to simply mimic their environment. Some animals, such as chameleons and anoles, have a highly developed background adaptation response capable of generating a number of different colours very rapidly.

Many fish have patterns of colours and tones that match their backgrounds so that they are indistinguishable even when plainly in view and can change by 2 main methods: physiologically or morphologically (Hinton 1976). Fish such as burrowing flatfish (Sumner 1911; Fujimoto et al. 1991; Reckel et al. 2002), flounder (Yamanome et al. 2004), sole and flatheads (Douglas and Lanzing 1980) can quickly adapt their colouration to match new backgrounds through a physiological change in colour. Physiological colour change is a fast response to a new environment and can occur over a period of a few minutes to a number of days. It is achieved by the dispersion and aggregation of melanin in the melanophores and gives the fish a lighter or darker appearance (hue) (Logan et al. 2006) but does not influence the overall body colouration (saturation and chroma) of the fish. Mid-water (pelagic) fish, like the lumpsucker (Davenport and Bradshaw 1995), seadragons and seahorses (Kuiter 2000) adapt more slowly by morphological colour change to match the kelp or seagrass in which they live in. Morphological colour change is a slower process in tissues where the quantity of specific chromatophores change and pigment content of chromatophores is altered in relation to the environment (Waring 1963; Bagnara and Hadley 1973).

Most fish, reptiles and amphibians undergo a limited physiological colour change in response to a change in environment. This type of camouflage is known as background adaptation. It has been demonstrated that the background adaptation process is triggered by the fishes vision and the reflectance of the near surroundings (Sugimoto 2002).
Very few studies have investigated the quantitative effects of background or light colour on fish colouration (Volpato and Barreto 2001). Adaptation to colour using lighting and background tank colour could be a solution to gain better market value in cultured or commercially farmed fish. Therefore this study will examine the effects of different background and light colours on the colouration of *H. abdominalis*.

2.1.1. Skin colour

The colour of skin is generated by the absorption, scattering and reflection of light. Light-scattering, light-reflecting organelles and coloured pigments in specialised skin cells, called chromatophores therefore create the body colouration of fish (Fujii 1993). Chromatophores are found in the dermis and can overlap and form layers. Chromatophores are grouped into subclasses based on their colour (hue) under white light: xanthophores (yellow), erythrophores (red), iridophores (reflective/iridescent), leucophores (white), melanophores (black/brown) and cyanophores (blue) (Matsumoto 1965; Bagnara 1966; Taylor 1969; Bagnara and Hadley 1973; Fujii 1993; Morrison 1995). Xanthopores contain large amounts of the yellow pteridine pigment and erythrophores contain carotenoids which make them appear red (Bagnara 1966; Fingerman 1970). Some chromatophores can contain both pteridines and carotenoids where the over all colour depends on the ratio of red and yellow pigments. Carotenoids are accumulated from the diet whereas pteridines are biosynthesised and then accumulated in the xanthophores (Bagnara 1998). Iridophores use crystalline schemochromes made of guanine (Taylor 1969; Morrison 1995) which reflect light and produce an iridescent bright blue or green colour depending on how the light is diffracted. Leucophores use crystalline purines which produce reflective white hues (Fujii 2000). Dark colours (e.g. black and brown) are caused by eumelanin in the

melanophores. Eumelanin is synthesised from tyrosine in a series of catalysed chemical reactions and packaged in vesicles called melanosomes and distributed throughout the cell (Ito and Wakamatsu 2003). Cyanophores which are responsible for a blue colour have been discovered recently. They contain a cyan biochrome of unknown chemical nature, and are rarely found in animals (Fujii 2000) except some fish species (e.g. cichlids Fig. 2.1).

There are two main mechanisms to aggregate and disperse pigments: by hormones or by neuronal action on chromatophores. Both methods can also act together to control the pigmentation shift (Sugimoto 2002).

Placidochomis phenochilus
with pigment colouration
(Cyanophores)

Protomelas taeniolatus
Fire Blue with structural colouration
(Iridophores)

Figure 2.1: Different blue colouration of cichlids (Franke-Rautenberg 2006).

2.1.2. Measurement of skin colour

One of the challenges of quantifying colour is how to measure it. There are very few colour standards used in fish colour definition. One of them is the Munsell colour

scheme which originally is used for soil colours and an other method is to use a portable spectrophotometer. The Munsell chart was not used because it is mostly seen as unsuitable for measuring animal colour. A portable spectrophotometer was not available and therefore a colour scheme based on the CIE (Commission Internationally d'Eclairage) Lab – colour space which is currently the most popular to measure animal colours (Weatherall and Coombs 1992; Stevens and Cuthill 2005) was chosen: the RAL DESIGN system™ (RDS). Its construction is not arbitrary but follows the CIE colour measuring system (RAL 2005b). It is a conversion of the XYZ-colour measuring system, and has the advantage that it orientates itself by the physiological qualities of the human perception: the chroma (instead of saturation) and the brightness (instead of remission) and not in physical measured values. Another advantage is the same visual spacing: the geometrically computable distances of two colour coordinates in the Lab – system correspond to the visually perceived distances, while in the XYZ-system the distances are geometrically bigger than the difference perceived by the eye with increasing chroma. The Lab – system takes the problems of the MacAdams ellipses into consideration, which makes it easier for objective assessment of colours as the axes in the Lab-space correspond directly to discernible qualities of the colours. Along the "a" axis red (+a) and green (-a) are distributed, while along the "b" axis yellow (+b) and blue (-b) values are found (Fig. 2.2) (CIE 1986).

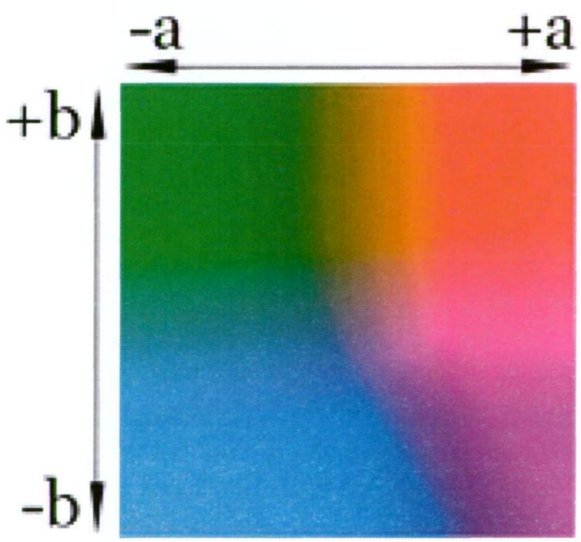

Figure 2.2: CIE Lab colour base at a lightness of 50% , with green (–a) and red (+a) on one axis and yellow (+b) and blue (-b) on the other (Wikimedia 2006).

The unchromatic colours are on the intersection of these axes, as well as the third axis, "L" which gives the brightness (Fig. 2.3). The colour coordinates of Lab – space definitions are not given in Cartesian coordinates (like the XYZ system), but in polar coordinates. Therefore the LCh-colour (XYZ) space corresponds to the Lab-colour space, the only difference exists in the coordinates where the colour is found (CIE 1986).

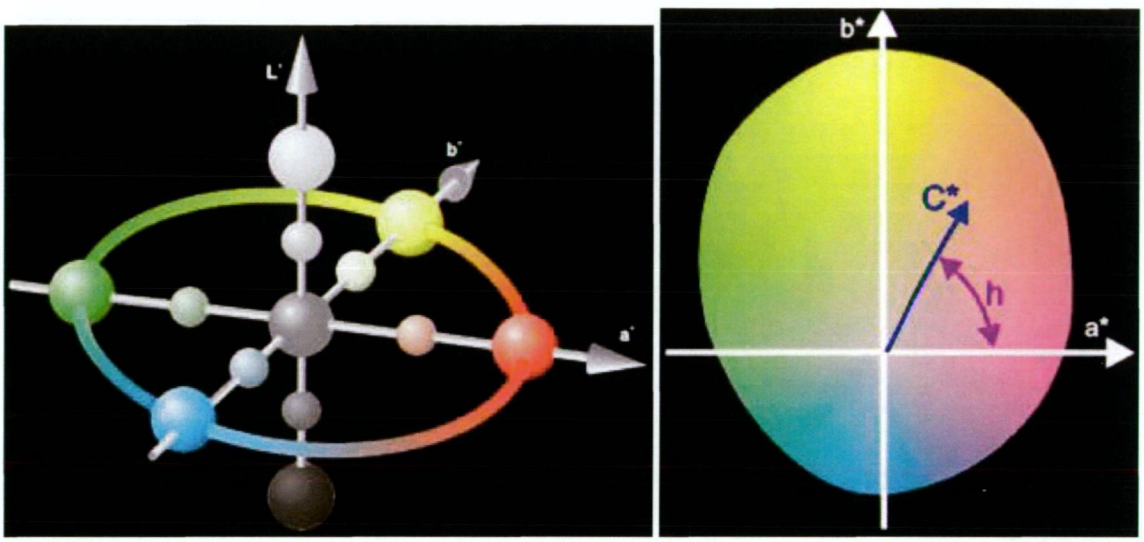

Figure 2.3: Drawings of CIE Lab colour space and LCh – colour space (Binder 2002).

In the RAL DESIGN system™ the colour distances between the individual colours are defined by the CIE Lab-colour distance formula. The colours of the RDS are organised systematically in hue, lightness and chroma values. Following the sequence of the colour spectrum, the hues are organised in a circle (Fig. 2.4). The designations correspond with the angles. Red can be found at 0° (= 360°), yellow at 90°, green at 180° and blue at 270°. The different values of lightness that are possible within each hue are arranged in various levels (Fig. 2.5). The non-chromatic axis runs through the centre and is synonymous with the scale for lightness. The non-chromatic axis starts with 0 at the bottom showing black, followed by continuously lighter greys ending with 100 on top representing white (RAL 2005a; b).

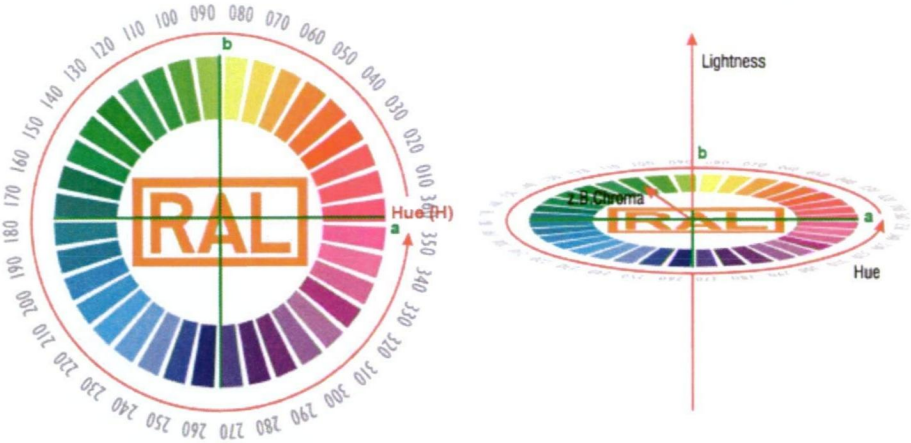

Figure 2.4: The RAL DESIGN system corresponds to the CIE Lab colour space. Each colour is represented in polar coordinates (angle from a central point; hue) and in cartesian coordinates (x – and y – axis lightness and chroma) (StudioDTP 2005).

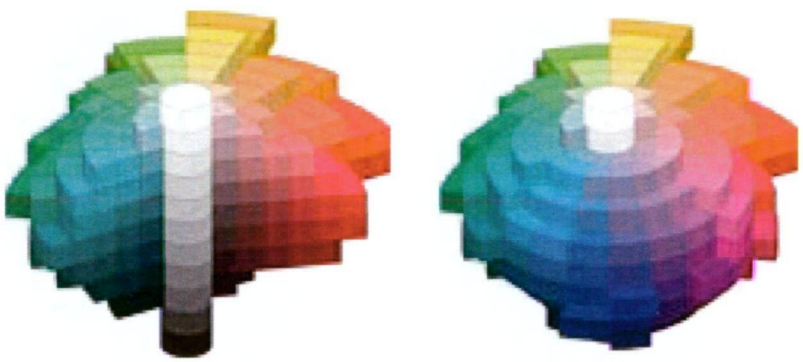

Figure 2.5: Part of the Colour Solid of the RAL DESIGN System with Non-Chromatic Axis and whole Colour Solid with all hues, values and chroma (RAL 2005a; b).

The first part of a colour code gives the angle in the colour circle, the second one the brightness, the third one the saturation. For example RAL 010 40 25: 010 = 10 ° angle in the colour circle, 40 = lightness/brightness, 25 = chroma/saturation (StudioDTP 2005).

This system was applied to determine the skin colour of the seahorses and the fish were colour coded by visually matching them with the colour charts under natural daylight.

An experiment was designed to examine if pot bellied seahorses (*H. abdominalis)* are capable of changing skin colour when exposed to either a specific background tank colour or light colour. Specifically the experiments aimed to test if any colour would promote a resultant golden-yellow colouration desired by the aquarium industry and if not to what extent any colour change does occur.

2.2 Materials and Methods

2.2.1 Experimental fish

Mixed sex juvenile seahorses (sexes could not be differentiated at this stage) were supplied by a commercial farm (Seahorse World Pty Ltd, Beauty Point, Tasmania). All supplied seahorses within one age group came from different breeding pairs and were pooled by age. Therefore parental genetic influences on colouration were unknown. All experimental fish were collected from the same tank to ensure the same preconditioning (light, tank colour) and age. Fish were transported to the research facilities of the School of Aquaculture at the University of Tasmania in 50 l containers supplied with oxygen (travel time = 45 min). Fish were temperature–acclimated from 16.6° C to 17.4° C (temp. diff. 0.8° C) for 15 min before random distribution between the 20 tanks in the background colour system and 15 tanks in the light system (20 fish per tank). The background and light adaptation experiments were conducted simultaneously using two separate tank systems.

2.2.2. Experimental systems

The background adaptation experimental system consisted of twenty square plastic tanks (33 cm x 30 cm x 33 cm) of five colours: red (040 40 67), yellow (085 60 60), green (160 50 60), blue (270 30 45) and white (semi–clear, 00 00) (n = 4). Each tank contained a weighted substrate for the fish to grasp, made from four 10 cm strands of a

poly–propylene rope of similar colour to the tanks (Red 030 40 60, Yellow 085 80 85, Green 140 60 70, Blue 260 40 45, White 00 00). Each tank had a water inlet positioned at the bottom and an outlet at the water surface. Water quality was maintained by a recirculation system comprising a pump, solids removal, biofilter and sump contained in a temperature and light controlled room (12L:12D, light on at 9.00 am). An airstone supplied each tank with additional aeration.

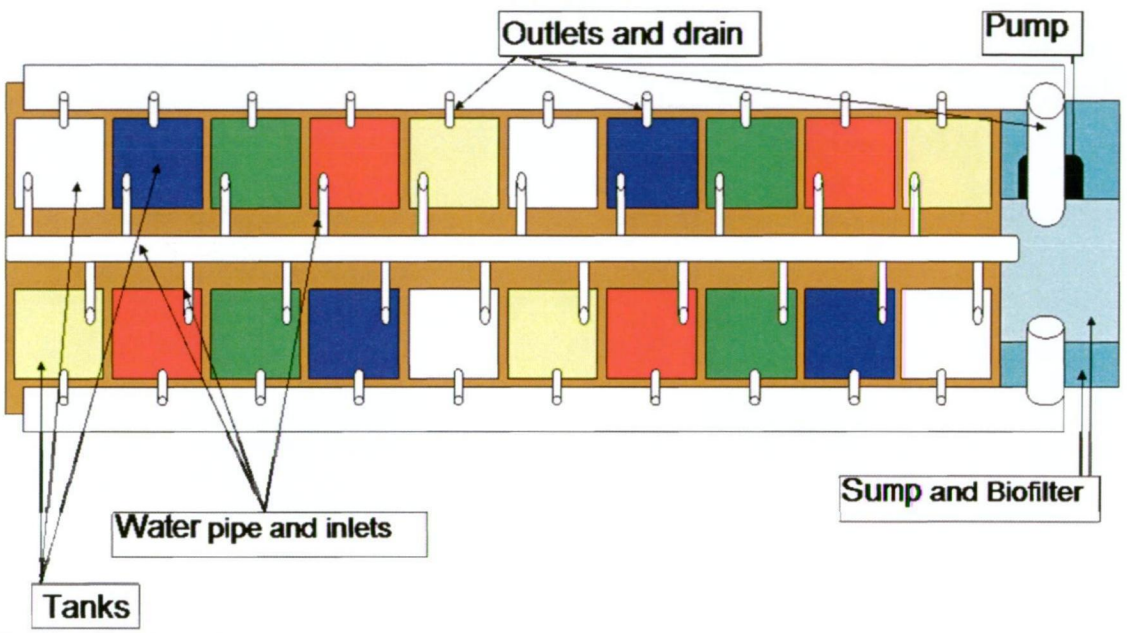

Figure 2.5: Schematic drawing (top view) of experimental design for background adaptation experiment.

The coloured tanks were systematically distributed as shown in Fig. 2.5 (yellow – white) and were illuminated by whole spectrum fluorescent lights (Luxeline plus F36W/850, Daylight deluxe, Sylvania, 3250 lm) above the tanks.

In the light adaptation experiment fifteen 20 l circular fawn coloured fibreglass tanks with a white base, which are commercially used in Seahorse farms, were illuminated by five different fluorescent coloured lights (Sylvania F36W/T8/: red (1250 lm), yellow (1580 lm), green (3140 lm), blue (700 lm) and Standard daylight F36W/154 (2500 lm); lm= lumen) (for spectra see Appendix C). One light illuminated three adjacent tanks from 30 cm above the water surface (Fig. 2.6). The tanks of one colour were separated

from others by black plastic. A white, circular plastic grid was placed in each tank as a substrate for the fish. Each tank had a water inlet positioned at the bottom and a screened outlet at the water surface. Water quality was maintained by a recirculation system comprising a pump, solids removal, biofilter and sump contained in a temperature and light controlled room (12L:12D). An airstone supplied each tank with additional aeration.

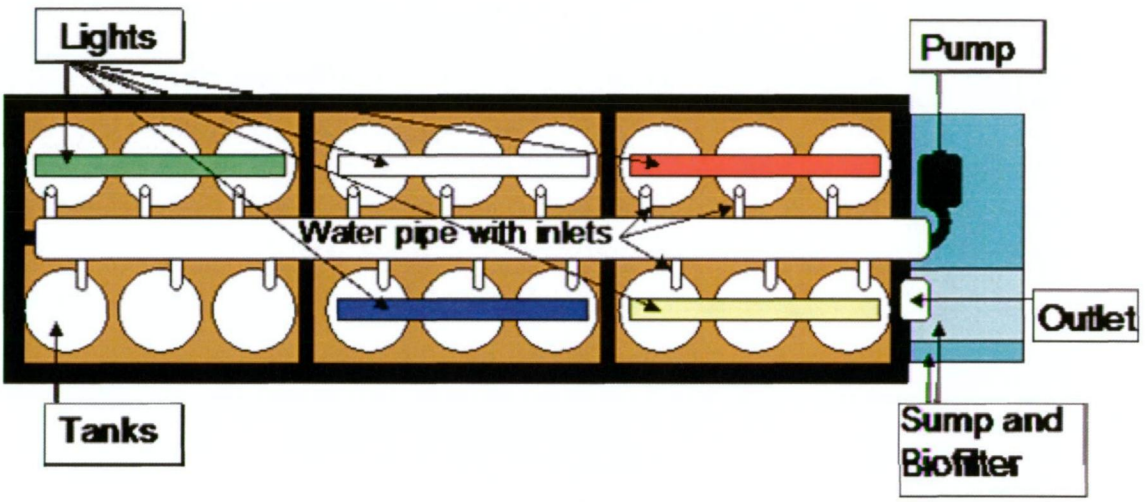

Figure 2.6: Schematic drawing (top view) of experimental design for light adaptation experiment.

2.2.3. Experimental protocol

Both background colour and light colour experiments were maintained under the same experimental protocols for a duration of 8 weeks. Colour assessments (totalling 5) were undertaken on individual fish at the start of the experiments, every 14 d and at the end of experiments.

Fish were fed with instar II *Artemia* once a day at 5% of their body weight (dry weight Artemia: wet weight fish) (Wardley 2001; Florent 2003; Woods 2005b). *Artemia* cysts were hatched in a hatching cone with aerated seawater at 28°C under bright fluorescent light. After 31 h the hatched nauplii were separated from cysts and harvested onto a 180

µm screen. Harvested *Artemia* were rinsed with fresh water and enriched in A1 DHA SuperSelco™ at a concentration of 0.3 g L^{-1} with aeration for 17 h prior to feeding the seahorses. Tanks were siphoned every day and cleaned regularly to remove surface fouling. Water quality (temperature, pH, salinity, ammonia, nitrite and nitrate) was checked twice a week with a colorimetric testing kit and was maintained at temperature of 16.37° ± 0.68°C (mean ± SD), salinity 34.32 ± 1.63 ‰ (mean ± SD), pH 8 ± 0 (mean ± SD), ammonia and nitrite < 0.5 mg l^{-1} and nitrate < 40 mg l^{-1}. Samples of fish (5 per tank) were measured for initial and final length (tip of tail to top of coronet) (± 1mm) and weight (± 1mg) to adjust feed rates during the experiment. Mortalities were recorded daily throughout the experiment.

Light intensity in each tank was measured underwater at the base of each tank using a data logger (Hoboware™ version 2 for Windows, Onset Computer Corporation; Intensity = Lux).

2.2.4. Skin colour assessment

To measure skin colour, all fish from one tank were individually anesthetised in benzocaine (100 mg l^{-1}) until the fish lost equilibrium. Fish were held against the RDS sheets and the colour which was most similar to the skin colours, of three body sections (ventral body, dorsal body and spots or stripes) (Fig. 2.7) under natural light was recorded. Fish were immediately placed in a well aerated bucket to recover and then placed back in to their respective tank. This procedure was repeated until all fish were measured.

To measure skin colour changes a modified RAL DESIGN system was used. The RDS was reduced from 1688 colours to the 415 most applicable colours (hue 060, 070, 080, 085, 100, 120, 140, 160, 200) for this experiment. To describe the range of skin

colouration, the charts were divided into four sectors. The divisions for the sectors were determined at a lightness of ≤ 60 and a chroma of ≤ 40. These values were chosen to produce sectors of dark – dull (s 1), dark – saturated (s 2), bright (s 3) and pale (s 4) colours (Appendix A1). The division were subjectively chosen initially and based on the yellow charts (080 and 085) as a reference.

2.2.5. Statistical analysis

Data for growth, survival and light intensity were assessed for normality and analysed by a one-way ANOVA (between treatments: growth, survival; within treatments: light intensity). Data were presented as means ± SE (or SD if specified).
Skin colour codes were recorded and transformed into separate numbers for hue, value and chroma by splitting the code into its respective components. Patterns in colouration were described separately for each body section (ventral, dorsal and spots/stripes) by multivariate ANOVA (MANOVA) (SPSS, version 12.0 (2003)) because of multiple pieces of independent information for each individual. Pillai's Trace was used to determine the significance level ($p < 0.05$) of differences between the skin colours of fish from different treatments. Discriminant analysis (DA) was used where significant differences in skin colour between different treatments were evident to describe where the differences lay. The summary of canonical discriminant (CDA) functions generated in DA expresses the number of independent variables (p) and the eigenvalues show how much of the variance in the dependent variable, is accounted for by each of the CDA functions.

The relative size of the function indicates the contribution to describe the variation present. To attach meaning to the functions the structure matrix was used. The structure matrix implies which variable is related to each of the functions in CDA. To classify the cases, the functions of group centroids were used where the cutting point is the weighted average of the paired values (Morrison 1967; Cooley and Lohnes 1971). Graphs were plotted in the software program SigmaPlot (version 10.0, Systat Software,

Inc. (2002)). Only the first two functions were used for the plot. The third function was rejected because the variance was less than 10%. Vectors will show the relative position and relation of hue, value and chroma towards the new created X– and Y– axes.

Figure 2.7: Ventral and dorsal body parts and spots of a fish which were used for the colour assessment.

2.3. Results

2.3.1. System 1: Background adaptation

Skin colour changes

Initial skin colour

There were no significant differences in dorsal, ventral and spot colour at the beginning of the experiment (day 0) for fish of both experiments. Both initial samples are represented in Fig. 2.8 (a) and show a slight variation in lightness and chroma for the ventral body part and spots which is seen as a natural variation. The fish were a uniform brown colour (dorsal) with minimal lighter brown ventral parts with no obvious spots (Fig. 2.8 a).

Skin colour after background adaptation

At the end of the background adaptation the diversity of colour in ventral, dorsal and spots had increased. Most fish had a dark ventral body colouration and fish from red and green tanks had the highest numbers of dark individuals (Fig. 2.8 b). Yellow and blue tanks produced more fish of a middle colour range (dark–saturated, see Appendix A1, s 2) than the three other colours. Yellow tanks produced yellow fish over a variety of hues (orange– to greenish–yellow) with 13.11 % of the fish having the market preferred golden–yellow colouration. Fish from white tanks were more uniform in colour being light greyish to greyish–white. Most pale fish were produced in the blue and white treatments with the highest numbers of fish in sector 4, hue 080 (Table A1 and Fig. A 2). The colouration of the dorsal region did not vary with any of the tank colours at d 56 and were of similar colour as at the beginning of the experiment (Fig. 2.8 a, b). Of the fish exposed to a blue or a white background only five showed a lighter dorsal colouration at the end of the trial (Fig. 2.8 b).

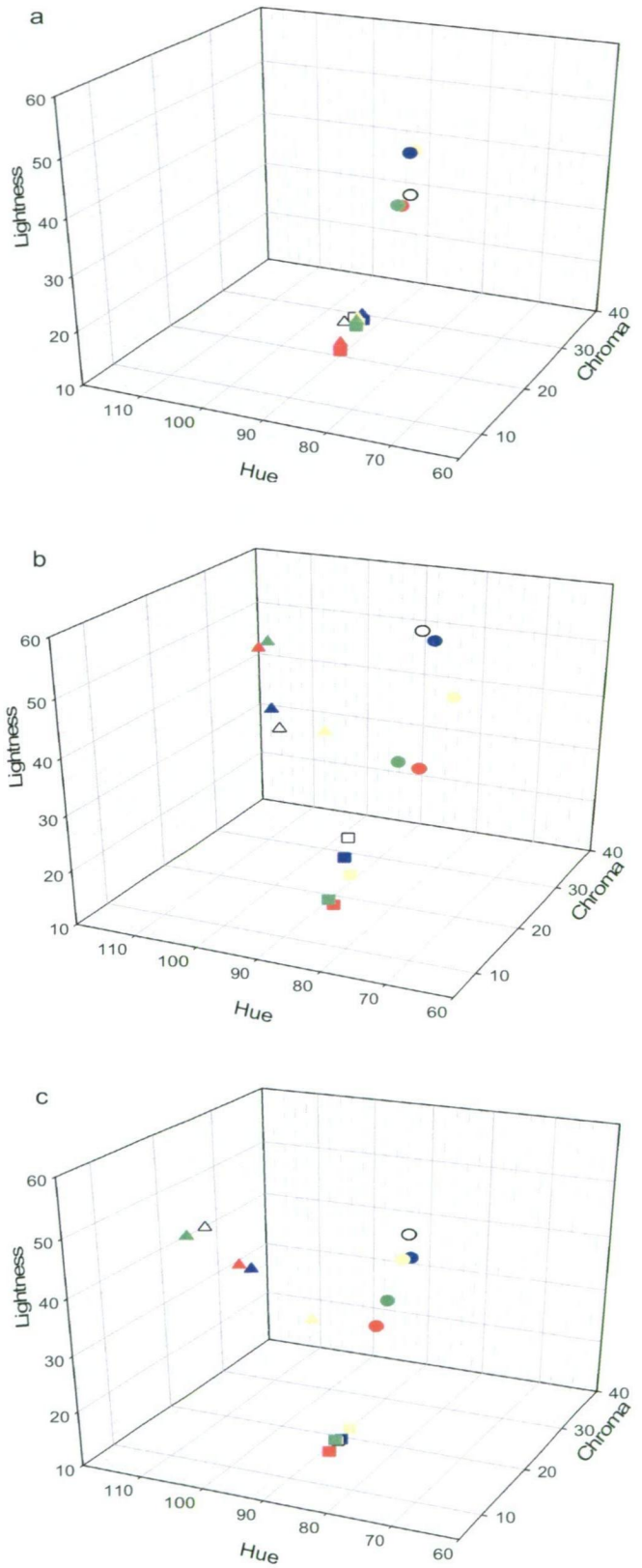

Figure 2.8: Mean skin colour at (a) day 0 (n = 700) and (b) day 56 for the background (n = 340) and (c) light colour adapted seahorses (n = 255). (● = ventral; ■ = dorsal and ▲ = spot colour)

Fish of red and green treatments had the highest variety of hues within spot colour (Fig. 2.8 b). Most of the red–adapted fish had pale greenish or dark spots. Amongst green–adapted fish the spot colouration varied from pale greyish–yellow over pale greenish and pale turquoise up to dark yellowish–brown (means ± SE: hue = 99.92 ± 3.76, lightness = 54.77 ± 2.40 and chroma = 19.54 ± 1.57 (see Table 2.1)). Blue and white adapted fish had either pale grey, grey–yellow or dark yellow–brown spots whereas fish held in yellow tanks had spots of a grey–brown to brown colouration.
Exact colourations of fish are listed in Tables A1 – A6 in Appendix A and can be cross–referenced with Fig. A1 and A2 to obtain detailed information about the fish's skin colour.
These visual observations of variation in colour patterns produced in each experiment were also described by the CDA/MANOVA shown in Fig. 2.9.

The ventral body part of the fish showed the most differentiation in response to background colour (Fig. 2.9). The x-axis (CDA 1) explained 85.8% of the variation, where the separation was driven by lightness (vector: v *). Fish of the treatment colours red, yellow and green were all darker in their appearance than fish from the blue and the white treatments. Fish from yellow tanks were more saturated and fish from green tanks were duller in the colouration of the ventral body part, described by CDA 2 (13.5%), while there was little variation in chroma (vector: c **) in fish of red, blue and white treatments. Yellow adapted fish were brighter and green adapted fish were less saturated than all other fish. Ventral hues were not statistically different among the treatments.

For the dorsal section of the body, the results were similar to those described for the ventral body parts. Most variance was described by CDA 1 (v *) which indicates that fish from red, yellow and green treatments were darker in value in their dorsal skin colour compared to fish of the blue and white treatments. Saturation (chroma) described on CDA 2 (16.1%) (c **) was more intense in fish from yellow treatments compared to all other adaptation colours. In relation to the spots most differences lay in lightness and

chroma of fish adapted to red and green compared to those adapted to yellow, blue and white tanks. Fish from red and green tanks were darker and less saturated in colour. The hues of the spots were also different in fish adapted to red or green, blue or white and yellow backgrounds. Red and green adapted fish had spots of green or turquoise hues whereas the spots of blue and white adapted fish were in the yellow-brownish range. Fish of yellow background adaptation had spots of orange and yellow hues.

Light intensities in the tanks

The light intensities in the tanks varied from 17 – 122 lx (Table 2.2) (0.22 – 1.46 µmol s^{-1} m^{-2}, Table 2.3) depending on the position of the light source above. The lights were fixed to the room ceiling and therefore could not be moved. The intensities of the four left hand side tanks were lower compared to the others due to this problem but had no significant influence on fish skin colour ($P = 0.913$, $f = 0.244$, $df = 4, 343$). In all other tanks light intensities ranged between 75 – 122 lx (0.90 – 1.46 µmol s^{-1} m^{-2}).

Table 2.1: Light intensities (lx) and approximate converted µ Einstein (µmol s^{-1} m^{-2}) (Biggs 1991) for all background adaptation tanks of different colours

Tank	W4	B4	G4	R4	Y4	W3	B3	G3	R3	Y3
lx	**18**	**45**	**91**	**118**	**86**	**122**	**87**	**86**	**79**	**75**
µmol s^{-1} m^{-2}	**0.22**	**0.54**	**1.09**	**1.42**	**1.03**	**1.46**	**1.04**	**1.03**	**0.95**	**0.90**
Tank	Y1	R1	G1	B1	W1	Y2	R2	G2	B2	W2
lx	**17**	**60**	**79**	**82**	**80**	**110**	**118**	**86**	**79**	**75**
µmol s^{-1} m^{-2}	**0.20**	**0.72**	**0.95**	**0.98**	**0.96**	**1.32**	**1.42**	**1.03**	**0.95**	**0.90**

Y= yellow; R= red; G= green; B= blue; W= white; 1, 2, 3, 4= replicant number

Growth and survival

No significant differences were found for initial length and weight or for length and weight gain (final length/weight – initial length/weight) at the end of the 56 days of the experiment (Table 2.3). The specific growth rate showed no influence of colour ($y = -0.2607\text{Ln}(x) + 0.9454$; $R^2 = 0.2939$; $n = 20$) or intensity ($y = 0.1653\text{Ln}(x) + 0.0337$; $R^2 = 0.1195$; $n = 20$) on growth. The survival of fish in all tanks ranged between 80 – 90% (Table 2.3) and was not significantly different between the tanks during the experimental period. Most of the mortalities occurred after handling the fish for colour coding indicating a possible effect of stress or effect of anaesthetic.

Table 2.2: Ventral, dorsal and spot colouration in hue, lightness and chroma for all seahorses at day 0 and day 56 (Mean ± SE).

Day 0		Ventral			Dorsal			Spots	
Colour	Hue	Lightness	Chroma	Hue	Lightness	Chroma	Hue	Lightness	Chroma
Red	79.49 ± 1.27	40.00 ± 2.07	19.37 ± 1.19	80.19 ± 0.30	22.28 ± 0.74	7.97 ± 0.40	80.44 ± 0.34	23.29 ± 0.85	8.35 ± 0.44
Yellow	78.91 ± 0.44	48.47 ± 2.21	21.26 ± 1.24	79.38 ± 0.33	25.40 ± 0.75	10.40 ± 0.63	79.67 ± 0.39	26.20 ± 0.92	10.69 ± 0.65
Green	79.54 ± 0.37	40.53 ± 2.07	18.60 ± 1.46	79.15 ± 0.60	25.42 ± 1.13	9.78 ± 0.86	79.30 ± 0.62	26.10 ± 1.20	10.05 ± 0.88
Blue	79.37 ± 0.40	48.21 ± 2.09	20.77 ± 1.22	79.03 ± 0.39	25.69 ± 0.80	10.98 ± 0.55	79.33 ± 0.45	26.44 ± 0.94	11.16 ± 0.55
White	79.43 ± 0.44	40.84 ± 2.05	21.13 ± 1.25	81.07 ± 1.25	25.15 ± 1.04	11.88 ± 0.93	82.14 ± 0.64	24.54 ± 0.99	11.23 ± 0.77
Day 56 background									
Red	77.78 ± 0.85	34.85 ± 1.74	21.77 ± 2.03	79.24 ± 0.45	20.91 ± 0.64	6.67 ± 1.01	102.65 ± 4.11	52.73 ± 2.65	20.83 ± 1.53
Yellow	77.14 ± 0.87	43.71 ± 2.29	28.43 ± 2.53	79.07 ± 0.60	24.00 ± 1.28	9.64 ± 1.05	87.64 ± 3.32	43.43 ± 2.83	15.50 ± 1.75
Green	78.69 ± 0.76	37.69 ± 1.99	18.77 ± 1.37	39.85 ± 0.33	21.85 ± 0.78	6.46 ± 0.48	99.92 ± 3.76	54.77 ± 2.40	19.54 ± 1.57
Blue	79.79 ± 0.56	55.34 ± 2.38	27.60 ± 1.89	79.57 ± 0.43	27.29 ± 1.64	9.14 ± 0.90	95.64 ± 4.06	46.29 ± 2.69	15.14 ± 1.37
White	81.30 ± 1.23	55.34 ± 2.29	27.07 ± 1.44	80.27 ± 0.14	29.45 ± 1.63	10.75 ± 0.96	93.56 ± 3.43	43.84 ± 2.88	14.18 ± 1.33
Day 56 light									
Red	80.47 ± 0.20	35.47 ± 2.29	15.28 ± 1.29	80.00 ± 0.00	20.57 ± 0.42	5.66 ± 0.33	100.38 ± 5.10	43.96 ± 3.31	13.30 ± 1.31
Yellow	79.70 ± 0.76	44.40 ± 3.20	19.60 ± 1.86	79.30 ± 0.50	22.40 ± 0.79	8.70 ± 0.81	88.20 ± 3.88	37.00 ± 3.22	12.60 ± 1.42
Green	80.44 ± 0.21	38.44 ± 2.50	17.56 ± 1.53	79.56 ± 0.42	22.22 ± 0.90	6.22 ± 0.40	108.44 ± 6.48	47.78 ± 3.27	13.06 ± 1.42
Blue	79.22 ± 0.61	44.14 ± 2.67	20.69 ± 1.48	79.22 ± 0.52	21.90 ± 0.62	6.90 ± 0.49	97.84 ± 4.95	43.97 ± 3.05	12.76 ± 1.26
White	80.11 ± 0.51	47.56 ± 2.92	21.44 ± 1.94	79.56 ± 0.44	21.78 ± 0.73	6.67 ± 0.59	108.56 ± 6.00	47.56 ± 2.90	16.24 ± 1.69

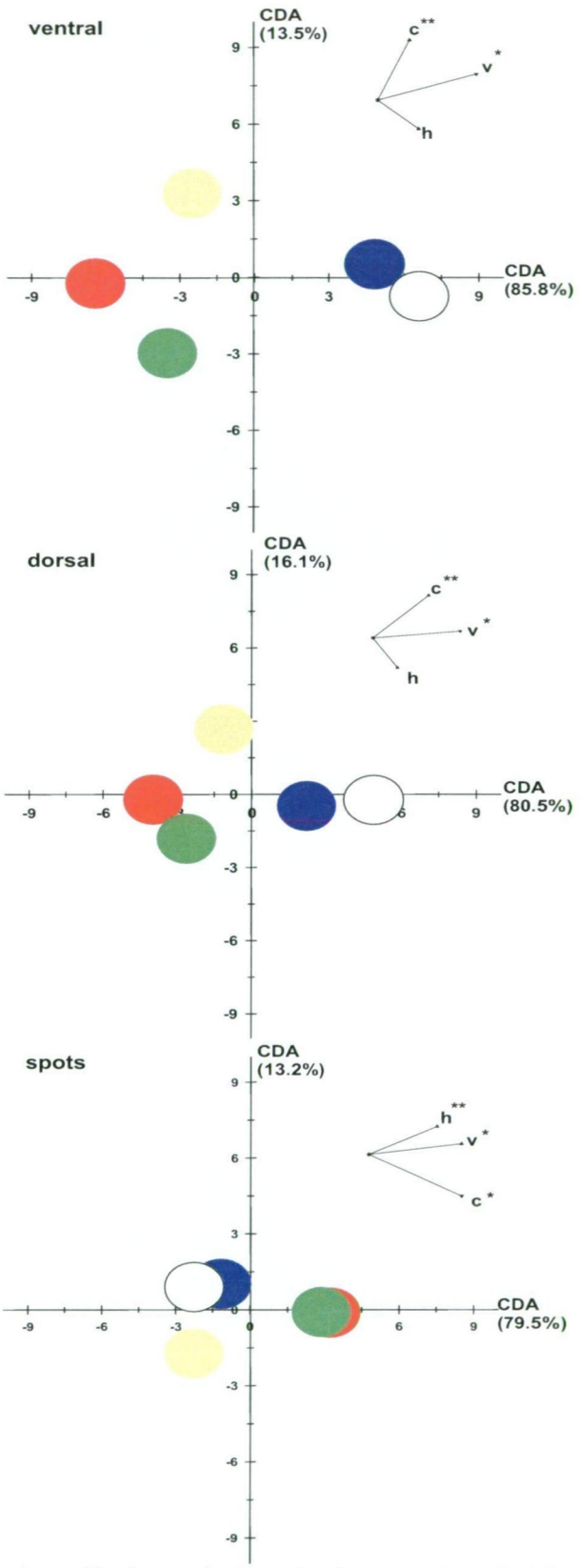

Figure 2.9: Skin colour of background adapted seahorses at the end of the experiment after 56 days. Centroids represent 95% of the seahorses in each treatment and CDA indicates the percentage of variance explained by the axes. Stars on the vectors (h = hue, v = value /lightness, c = chroma) show their position relative to the X – (*) and Y- axes (**).

Table 2.3: Growth performance and survival of seahorses reared under different coloured backgrounds. Values are means ± standard error for all seahorses from replicates of the same colour. (df 4, 19)

Tank colour	red	yellow	green	blue	white	ANOVA F	ANOVA P
Initial weight (g)	1.00 ± 0.01	0.97 ± 0.02	0.91 ± 0.02	0.89 ± 0.02	1.01 ± 0.01	1.68	0.208
length (cm)	7.15 ± 0.08	7.18 ± 0.11	7.44 ± 0.06	7.69 ± 0.08	7.46 ± 0.12	1.19	0.353
weight gain (g)	0.47 ± 0.08	0.46 ± 0.08	0.58 ± 0.08	0.53 ± 0.11	0.43 ± 0.11	0.38	0.819
length gain (cm)	1.65 ± 0.18	1.55 ± 0.48	1.36 ± 0.33	1.33 ± 0.40	1.35 ± 0.24	0.17	0.949
SGR (% day)	0.68 ± 0.11	0.69 ± 0.13	0.87 ± 0.12	0.83 ± 0.17	0.62 ± 0.13	0.66	0.629
Survival (%)	82.50 ± 1.28	86.25 ± 0.58	80.00 ± 1.61	86.25 ± 0.90	90.00 ± 1.08	0.68	0.617

$SGR = (\ln_{\dot{\omega}2} - \ln_{\dot{\omega}1})/t_2\text{-}t_1) * 100$ ($\dot{\omega}$ =weight, 1 = initial, 2 = final, t_1 = first day of exp., t_2 = last day of exp.)

2.3.2. System 2: Light adaptation

Skin colour changes

Initial skin colour

The skin colour of fish for the light adaptation experiment was considered similar to the fish of the background adaptation experiment (Fig. 2.8 a) as they were selected from the same stock.

Skin colour after light adaptation

Fish in the light adaptation experiment changed their body colouration to a lesser extent as displayed in Fig. 2.8. White, green, red and blue treatments produced some fish with pale greyish spots. There were few ($n \leq 9$) pale fish across all light treatments (for exact numbers see Appendix 1). No fish with a market–preferred golden–yellow colouration was observed in the light experiment.

Yellow, blue and white treatments produced some fish with a pale and light saturated ventral body part but most fish remained with a dark colouration (Fig. 2.8 c). Colouration for the dorsal part of the body was dark in all fish similar to the start of the experiment.

Fish showed some variation in spot colour where the hues of the initial fish ranged between 080 and 085 (mean ± SE: 79.30 ± 0.62 to 82.14 ± 0.64) (Fig. 2.8 a and Table 2.2) and broadened to hues of 080 – 200 at the end (mean ± SE: 88.20 ± 3.88 to 108.56 ± 6.00) (Fig. 2.8 c and Table 2.2). Fish with lighter spots were mostly found in green and white treatments with few in red and blue treatments. Spot colouration of fish from yellow treatments remained fairly dark but was slightly lighter than at the beginning of the experiment (Fig. 2.8 and Table 2.2).

Statistically the skin colours in light adapted fish only varied significantly for the dorsal body part (Fig. 2.10). The variability of colour for the ventral parts and spots was very high and therefore no statistical differences were found by ANOVA. Yellow – adapted fish statistically had a much lighter and more saturated dorsal colour than fish of the other four treatment colours which is explained by CDA 1 (83.9%), representing the chroma component. However the variation in chroma of the colour yellow was biologically minimal and was influenced by the nature of the colour yellow, which is brighter than all other colours and therefore was described by higher numbers in the colour measuring system.

The lightness of the dorsal skin colours which is described on the Y-axes (variance of 14.5%) was caused by single individuals of the red and green treatments and overall the majority of fish from these treatments did not differ greatly in lightness.

Light intensities in the tanks

Light intensities were similar between the three tanks of each lighting colour treatment. Green light had the highest intensity with a mean ± SE of 138.67 ± 3.18 lx (1.66 ± 0.04 μmol s^{-1} m^{-2}), followed by red light with 102.67 ± 0.67 lx (1.23 ± 0.01 μmol s^{-1} m^{-2}), white light with 70.67 ± 0.67 lx (0.85 ± 0.01 μmol s^{-1} m^{-2}), yellow light with 59.34 ± 2.91 lx (0.71 ± 0.03 μmol s^{-1} m^{-2}) and the blue light with a mean intensity of 30.34 ± 1.67 lx (0.36 ± 0.02 μmol s^{-1} m^{-2}).

Table 2.4: Light intensities in lux (lx) and µ Einstein (µmol s^{-1} m^{-2}) (Biggs 1991) for all light adaptation tanks of different colours

Tank	G3	G2	G1	W3	W2	W1	R3	R2	R1
lx	**136**	**145**	**135**	**70**	**70**	**72**	**102**	**102**	**104**
µmol s^{-1} m^{-2}	**1.63**	**1.74**	**1.62**	**0.84**	**0.84**	**0.86**	**1.22**	**1.22**	**1.25**
Tank				B1	B2	B3	Y1	Y2	Y3
lx				**27**	**32**	**32**	**60**	**64**	**54**
µmol s^{-1} m^{-2}				**0.32**	**0.38**	**0.38**	**0.72**	**0.77**	**0.65**

Y= yellow; R= red; G= green; B= blue; W= white; 1, 2, 3, 4= replicate number

Growth and survival

No significant differences were found for initial length and weight or for length and weight gain (final length/weight – initial length/weight) at the end of the 56 days of the experiment (Table 2.5). The specific growth rate (SGR) shows that there were no differences in growth caused by any of the treatment colours ($y = -0.1555\ln(x) + 1.1608$; $R^2 = 0.0953$; $n = 15$) or intensities ($y = 0.2219\ln(x) + 0.1222$; $R^2 = 0.2558$; $n = 15$). The survival of all tanks ranged between 86.67 – 96.67% and showed no significant differences between the tanks (Table 2.5) during the adaptation period. None of the lighting colours appeared to have any significant effect on the survival of the fish and most of the mortalities occurred after handling the fish for colour coding indicating a possible effect of stress.

Fehler! Keine gültige Verknüpfung.

Figure 2.10: Dorsal skin colour of light adapted seahorses at the end of the experiment after 56 days. Centroids represent 95% of the seahorses and CDA indicates the percentage of variance related to the axes. Stars on the vectors show their position relative to the X – (*) and Y–axes (**).

Table 2.5: Growth performance and survival of seahorses reared under different coloured lights. Values are means ± standard error for all seahorses from replicates of the same colour. (df 4,14)

						ANOVA	
Tank colour	red	yellow	green	blue	white	F	P
Initial weight (g)	0.70 ± 0.02	0.73 ± 0.00	0.73 ± 0.00	0.85 ± 0.03	0.77 ± 0.01	1.50	0.273
length (cm)	7.16 ± 0.06	7.21 ± 0.02	7.36 ± 0.09	7.59 ± 0.09	7.37 ± 0.04	1.36	0.314
weight gain (g)	0.59 ± 0.11	0.62 ± 0.16	0.71 ± 0.07	0.48 ± 0.07	0.76 ± 0.07	1.09	0.412
length gain (cm)	0.64 ± 0.25	1.13 ± 0.36	1.24 ± 0.13	0.88 ± 0.14	1.51 ± 0.05	2.34	0.126
SGR (% day)	1.08 ± 0.11	1.06 ± 0.20	1.17 ± 0.06	0.80 ± 0.11	1.22 ± 0.09	1.71	0.224
Survival (%)	82.50 ± 1.28	86.25 ± 0.58	80.00 ± 1.61	86.25 ± 0.90	90.00 ± 1.08	3.17	0.063

$SGR = (\ln_{\acute{\omega}2} - \ln_{\acute{\omega}1})/t_2\text{-}t_1) * 100$ ($\acute{\omega}$ =weight, 1 = initial, 2 = final, t_1 = first day of exp., t_2 = last day of exp.)

Algae growth

After the first week of the trials a quite dense algal growth was observed in all of the tanks but the colour of the algae were different under different coloured lights. Samples were taken, using sterile swaps, on the same day as the second skin colour measurement and the tanks then cleaned. The samples were placed on microscopic slides under cover slips and examined by light microscopy. The examination showed that all tanks contained the same algal groups (cyanobacteria, diatoms) but in different ratios. The tanks with red and yellow light had a higher proportion of cyanobacteria that probably synthesised blue-green pigments and a low proportion of diatoms with a green – brownish colour. In the other three tanks with green, blue and white light the dominant cyanobacteria synthesised red pigments (phyco-erythren) and the fouling community appeared an overall dark red colour. A higher proportion of diatoms was also observed in these tanks adding a brownish appearance.

In summary the results of these adaptation experiments have shown that it is possible to change the skin colour of pot bellied seahorses *H. abdominalis.* Best results of achieving the market–preferred golden–yellow colouration were obtained by exposure to yellow coloured backgrounds for a period of 56 days. Adaptation to the background colours red and green resulted in colour morphs (dark with iridescent green or turquoise spots) not described previously while white tanks produced pale white to ivory coloured fish. This experiment has also shown that lighting colour does not have a great influence on the skin colour of seahorses as the adapted fish did not change their skin colour in response to the lighting colour they were exposed to.

2.4. Discussion

2.4.1. Background adaptation

The light intensity range (75-122 lx) measured under experimental conditions (see table 2.2) resembled natural light levels at the ocean surface at dawn and sunset (Nicol 1989). The low levels of light intensity in four of the experimental tanks did not influence the fish to adapt to their given treatment colour. Yellow and white adapted fish changed their skin colour to a higher degree with respect to their background colour, than did fish of other colour treatments. The golden-yellow colouration preferred by markets (Vincent 1994; Wardley 2001) was predominantly achieved within fish from yellow background treatments (Fig. 2.8). Meyer (1931) reported that sole and gobies cultured on yellow backgrounds also tended to adopt a more yellow skin colouration. Fish from white tanks were mostly light yellow–greyish in skin colour with a less saturated yellow than yellow–adapted fish (see Fig. 2.8). Therefore fish held in both the yellow and white tanks appear to have undergone a morphological colour change in which the melanophores were reduced and the numbers of xanthophores and leucophores probably increased (Waring 1963; Bagnara and Hadley 1973; Fujimoto et al. 1991; Stuart et al. 1996). Alternatively the number of these chromatophores may have stayed the same but the concentration of pteridine and purine within the chromatophores was much higher. Both ways of changes in the cellular structure are possible but further studies have to be undertaken to examine the mechanism of morphological colour change in this species.

Spots and/or stripes often help animals make them more invisible to enemies (Hinton 1976). Red and green adapted fish remained dark brown on the ventral and dorsal body but both colour treatments produced fish with iridescent green and turquoise spots which in case of the green treatment seemed to be a form of adaptation (Fig. 2.8). The reason for red–adapted fish also developing green or turquoise spots is unclear, but could have several explanations. It could be that because red (ca. 580 nm – 800 nm) and green (ca. 480 nm – 560 nm) are very close at the higher end of the visual spectrum that fish perceive red as green in their tanks, and therefore both display a green-bluish skin

colouration in their spots. Another reason could be that the red tank background did not reflect as much light as the other background colours and therefore the stimuli for the fish was not sufficient to produce higher numbers of erythrophores. The light reflection from the background which indirectly reaches the fish's eye plays a fundamental role in governing the perception and response to background colour (Bagnara and Hadley 1973). Adapting the colour of the spots to the background colour gives fish a disruptive colouration (Cott 1940) which in nature makes them more invisible and probably is more energetically efficient than trying to match the whole body colour to the background. *H. abdominalis* with green or turquoise spots have never been described before but could possibly be of high value if introduced to the commercial market.

Fish held in blue tanks displayed light greyish-brown ventral and dorsal body colours with brown spots (Fig. 2.8). This suggests that the fish reacted to the blue background by the dispersion of melanin and a build up of purines in the leucophores in their ventral and dorsal body parts. As no pigmented blue body colouration was observed during the adaptation period, cyanophores may have been absent in this species.

Background adaptation can not only cause a change in body colouration but can also influence fish growth and survival. Dark tank colours seem to reduce growth (Papoutsoglou et al. 2000; Papoutsoglou et al. 2005) or even cause lower survival (Downing and Litvak 1999; Pedreira and Sipauba-Tavares 2001) in some larval fish due to poor food particle contrast to the background and higher stress levels. However for some species the opposite may be the case depending on their natural visual ability (Duray 1995; Jentoft et al. 2006). In this experiment growth and survival was not significantly influenced by any of the background colours (see Tables 2.3) suggesting that background colour had no or minimal effects on functions such as stress and prey intake. Such an effect has been shown by Martinez – Cardenas and Purser (2007) in early juvenile *H. abdominalis*.

2.4.2. Light adaptation

Coloured light had a minimal influence on the skin colour of *H. abdominalis*. Kunz (1920) exposed eggs and larvae of the sole, *Pleuronectes spp.*, and of the pike, *Esox lucius*, to light of five different colours and showed that the body colouration of pike was not affected, but that sole larvae adapted to red, yellow and green light produced a more yellowish body colouration. Fish adapted to light colours in the present study, mainly stayed in the natural colour range (yellowish–brown) (see Fig. 2.8). The significant difference in dorsal colouration for yellow–adapted fish (Fig. 2.10) was not supported by observations and is related to the colour yellow, which naturally has lighter than other colours. Light colour does not have the same influence as background colouration on the skin colouration of cryptic species of fish (Sumner 1940). The lack of differentiation in the fish colour suggests that the effect of stimuli of direct coloured light may not be as strong as the reflecting light from coloured backgrounds. The white tanks reflect all light (Waring 1963) and although the intensities of the red, yellow and white light were in a similar range for the background and light adaptation system, the angle of light reflection from different shaped tanks (square, cylindric) could have been perceived differently by fish and have minimised the stimulus for skin colour adaptation to different coloured lights.

Coloured light has been shown to influence growth rates of Atlantic Salmon, *Salmo salar* (Stefansson and Hansen 1989), Haddock, *Melanogrammus aeglefinus* (Downing and Litvak 1999), Crucian carp, *Carassius carassius,* Chinese sleeper, *Perccottus glenii* and Guppy, *Poecilia reticulate* (Ruchin 2005) and stress response (Volpato and Barreto 2001) of fish, where green and blue light resulted in higher survival, growth rates and less stress in juvenile and adult fish. In contrast, none of the lighting colours in the light adaptation experiment of *H. abdominalis* had a significant influence on growth or mortality.

The ability of the fish to change colour may have also been influenced by genetics (combined broods) where the size of the gene pool could reflect the ability of some fish

to change their body colouration to a greater extent than others regardless of which system they were reared in.

Overall these findings show that there are possibilities for the commercial growers to grow fish to suit the market requests of more colourful *H. abdominalis* and particularly yellow fish. Golden–yellow coloured fish could probably be supplied to the market if fish are held in yellow tanks over longer periods of time or maybe even breed in this tank colour. Of further interest could also be the fish with a dark body colouration and green and turquoise spots, which were produced in red and green tanks or white fish from the white tanks. This could be evaluated by the commercial farms and market research undertaken to examine the potential of sales for fish with these colourations.

The relationship between colour perception and skin colour displayed in seahorses needs to be examined in further studies to understand why background and light adaptation to certain colours achieves a skin colour change and other colours do not. Therefore evaluation of skin pigments before and after adaptation could be interesting as well as measurements of retinal pigments with a microspectrophotometer (MSP).

Chapter 3

Colour preferences of the pot bellied seahorses *Hippocampus abdominalis* before and after adaptation to different experimental conditions

3.1. Introduction

3.1.1. Light as an important factor in life of fishes

Light can play an important role in the life of seahorses especially on their stress levels/ health, reproduction, feeding and locomation. Most species of seahorses exhibit certain behaviours as simple greeting, courting and mating at the time of sunrise (Kuiter 2000). The ability of seahorses to detect light depends on penetration of the light through water and the spectral qualities of the retinal pigments, which work as light receivers (Simenstad et al. 1999). The retinal arrangements of photoreceptors and their spectral sensitivity will be tuned to a greater or lesser extent to the specific photic conditions of the fish's habitat (Bowmaker 1995).

At the surface of the water, radiation is reflected by the sun on a partial spectrum which lays around 300 nm in ultraviolet up to about 1100 nm in the infrared area (Fig. 3.1). Below the water surface the spectrum of available light is further limited.

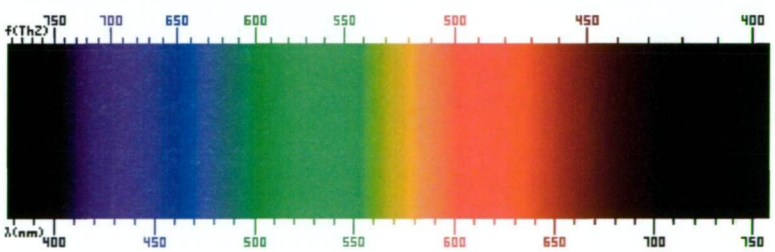

Figure 3.1: Visible light and its wavelengths: from short wavelengths: blue to long wavelengths: red.

Water serves as a monochromator which absorbs long– as well as short–wave light with a maximum transmission in pure water at around 460 nm in the blue region. However, natural water bodies are seldom pure and may contain many impurities such as suspended particles, which will scatter short wavelengths and phytoplankton and dissolved substances which can colour the water. In clear oceanic waters the reduction of light is minimal and the limit of the photopic vision is reached at depths of 300-500 m, where the maximum transmission is approximately 470 nm. In coastal waters the photic zone is reduced to 30-50 m due to high levels of suspended particles, and subsequently the maximum transmission is displaced to longer wavelengths of 530-570

nm. The photic environment is a main factor affecting the visual system of fishes, and fish adapt and specialise their vision to their habitat (Bowmaker 1995).

3.1.2. Colour vision of fishes

Fishes possess colour vision, which is made possible by specialised structural elements in the retina. The rods and cones, which serve as photoreceptors are in the front layer of the retina. The number of rods and cones differ according to the life history of the fish species. Day-active fishes have predominantly cones and nocturnal fishes which feed during dusk or at night posses mainly rods. For example, in the same part of the retina, the nocturnal burbot *(Lota lota)* has 260 rods and the day-active pike *(Esox lucius)* has 18 rods. In bright light (photopic), the cones contract and lie close to the external limiting membrane, the rods elongate and the pigment shifts downwards to the cones outer segments (Fig. 3.2 A) and in darkness (scotopic) the cones elongate, the rods contract and the pigment moves back into the bases of the epithelial cells (Fig. 3.2 B) (Nicol 1989).

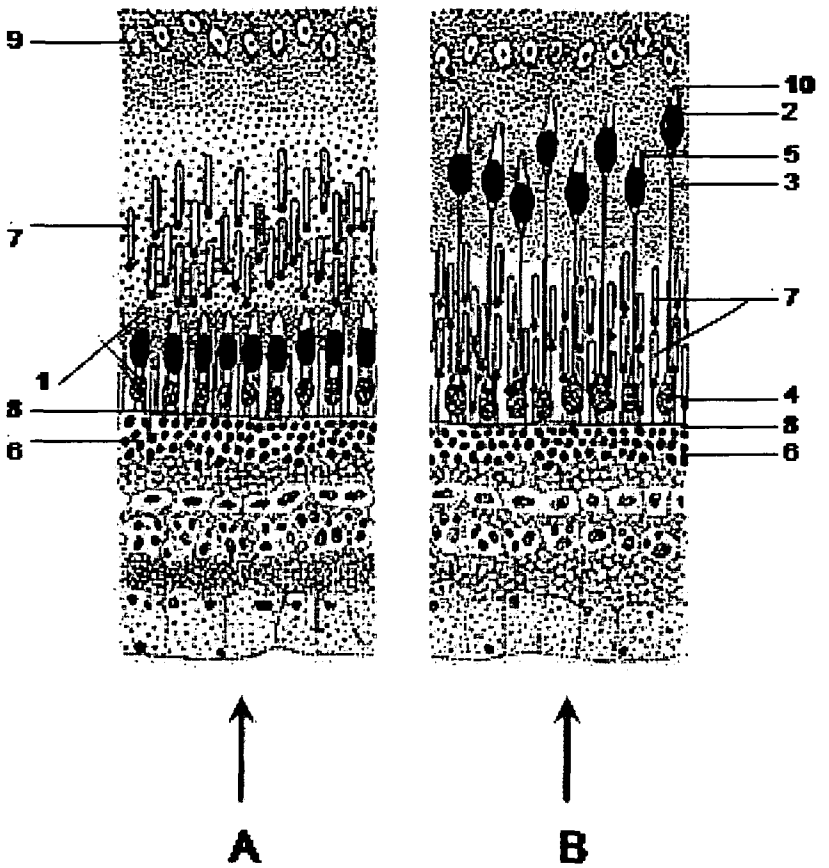

Figure 3.2: Retina of the bream carp (*Abramis brama*) A photopic and B scotopic: 1 cones; 2 cone ellipsoid; 3 cone myoid; 4 cone nuclei; 5 cone outer segment; 6 outer nuclear layer; 7 rods; 8 external limiting membrane; 9 nuclei of the pigment epithelium; 10 pigments. Arrows show light induction (Suworow 1959).

Cones are responsible for colour perception, and are present as single, double, triple and even quadruple cones. The bony fishes (Teleosteii) have single and double cones except for a few species like the brown trout (*Salmo trutta*) which has triple cones (Suworow 1959; Bowmaker and Kunz 1987). Many fishes are dichromatic (two colour vision) and have cones which are sensitive to blue and green light wavelengths, due to their construction and pigmentation (Suworow 1959; Nicol 1989). Cyprinids are tri– or tetra–chromatic and likewise have cones, which are red or yellow sensitive. Some species show a clear correlation between cone morphology and spectral orientation, e.g., the goldfish (*Carassius auratus*) and the brown trout have blue sensitive single cones and red/green sensitive double cones (Bowmaker and Kunz 1987). The existence of colour

vision in fish affects the ability to detect colour and therefore their feeding habits and their behaviour.

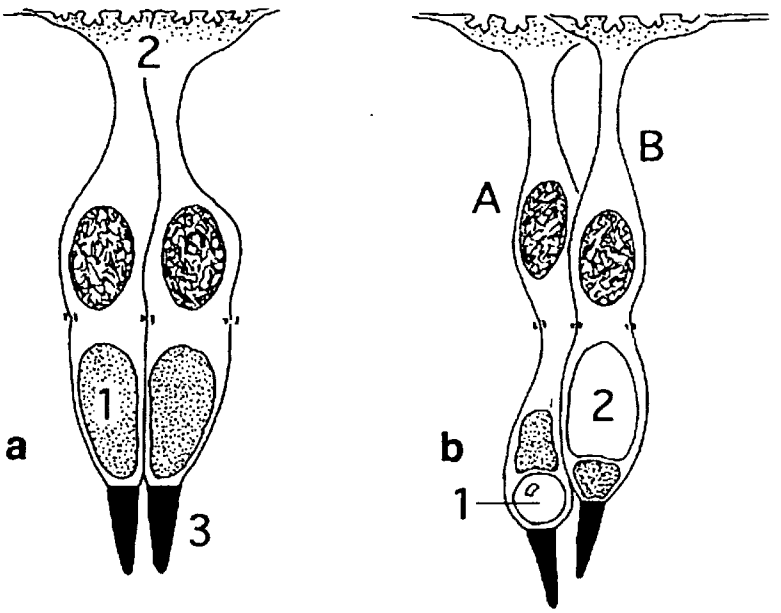

Figure 3.3: Different double cones: a: twin cone, as found in teleosts. The ellipsoid of the two members are in close contact; 1: pedicle, 2: outer segments; 3: ellipsoid. Although they look identical they may contain different visual pigments. b: double cone, as in reptiles, amphibians and birds. The chief cone, A: has an oil droplet; 1: but no paraboloid. The accessory cone; B: has no droplet but a large paraboloid; 2: conducting fibres and pedicles are separate, but inner segments are in close contact (Locket 1999).

3.1.3. Behavioural reactions to coloured objects

Fish can be attracted by coloured objects in their surrounding like plants, corals or even floating debris. Behaviour of fish associated with coloured "Fish Aggregating Devices" (FADS) was demonstrated in a study by Kawamura (1996) in which fishes showed a preference for the blue and green FADS but kept a distance of a few metres from the white FAD.

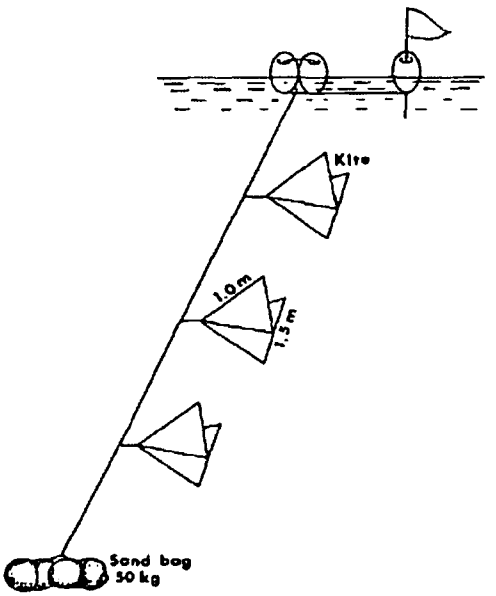

Figure 3.4: Structure of the experimental FADS used by Kawamura (1996).

Fish tended to be least abundant near the black and white FADS. The yellow and red FADS were visited with a middle frequency. The divers were able to distinguish the colour of each kite to a depth of 15 m, although the colours of the kites 'faded' with water depth and red became dark brown below 10 m. The underwater visibility of the kites varied with water transparency and lighting conditions. The yellow kite was always the most visible followed by the white kite, as a result of high radiance from them. The green kite was the least visible at the experimental site in Japan (Kawamura et al. 1996).

Colour perception under water depends on the visual acuity of the observers eye, the spectral qualities of the underwater light and on the reflection of the object. The chromatic spectrum of the visual perception of the coastal fishes is similar to that of human beings (Kobayashi 1962).

Fish seem to prefer blue and green which was examined in several experiments. Muntz and Cronly-Dillon (1966) demonstrated, that the goldfish (*Carrasius auratus*) is more attracted to blue and green objects than to red. In a free colour choice test Kawamoto and Takeda (1950) reported a preference for blue and green of the Japanese parrotfish (*Oplegnathus fasciatus*) and for seven other Japanese marine fishes. Studies of brown trout fry showed their preferences for green (Maaß 2004). In the studies of Kawamoto (1950; 1951) and Maaß (2004) fish could freely swim into sectors or compartments in

which different colours of light were applied and preferences were calculated on numbers of visits per colour.

In aquaculture of new species like seahorses, where no colour preference studies have been performed, tank colour and lighting is expected to affect the vision and behaviour of the fish. Therefore this study aimed to examine the natural colour preferences of the pot–bellied seahorses during different stages of development and after an 8 wk adaptation period to different holding conditions. It was also an aim to examine which holding factors (tank colour/ lighting colour) have a greater impact on changing colour preferences of the pot bellied seahorses.

3.2. Materials and Methods

3.2.1. Experimental fish

Seahorses of different ages and different holding conditions were tested for effects of life history, ontogeny or acclimation on their colour preference (see Chapter 2: 2.2.2. and 2.2.3.). Fish used in the colour preference tests were either from the existing stock of the School of Aquaculture or the fish from Chapter 2 (2.2.1.). The school kept different groups (age, size) of seahorses in either light grey or light blue special seahorse tanks (broodstock, older fish) or in 20 l circular fibreglass tanks (fawn sides, white base) (young fish) with white (Luxeline plus F36W/850, Daylight deluxe, Sylvania, 3250 lm) overhead illumination (12L:12D) in a temperature controlled room (around 16°C). All fish from the school's stock were counted and their ages identified and if there were 100 individuals the group was chosen for the colour preference tests (2, 7, 9 and <18 month old). The group of 5 month old fish was from the fish used in Chapter 2 at day 0 as well as all fish with colour pre–treatments (after day 56).
The school's stock was reduced before a long holiday period for reasons of daily maintenance and therefore just the group of over 18 month old fish could be tested in the background colour preference test.

3.2.2. Experimental systems

A colour preference test (Maaß 2004) where five colours were tested simultaneously (Fig. 3.5 – 3.7) was used to examine if seahorses prefer different coloured tank environments.

Background preference tank configurations

The preference testing tank was a circular 100 l tank with five timber partitions (with white aqua enamel painted surface) positioned to give five compartments of identical

size with access to a central cylindrical compartment (Fig. 3.5). The internal surface of each compartment offered a different colour background (red (030 50 60), yellow (085 80 85), green (180 30 35), blue (270 30 45) and white (100 90 05) (coded with RAL Design)) and a white fluorescent tube (Sylvania®, 36W) illuminated the tank from above. Coloured contact paper was used on the tank surface because paint would not attach to the plastic and the paper was non-toxic for the fish. The middle tube was covered with a polystyrene lid to darken the inside.

Light preference tank configuration

The tank configuration was identical to the coloured background preference test tank except the five areas were illuminated with the different test colours (Chapter 2: 2.2.2.); (red = 1250 lm, yellow = 1580 lm, green = 3140 lm, blue = 700 lm, white (whole spectrum) = 2500 lm) using fluorescent lights of 36 W (Sylvania 2004) The central compartment was not provided with any overhead lighting. The coloured lights were randomly distributed and the position changed after each test. During each test 10 fish were released into the central compartment, from which they could freely move to the compartment of preferred colour (Fig. 3.6).

3.2.3. Experimental protocol

Fished were classified into three groups: non–adapted (school's stock), background–adapted and light–adapted fish (Chapter 2: 2.2.2.). Non–adapted (light grey or white background and white light) fish tested in the light preference test were 2, 5, 7, 9 and over 18 months old and for the background preference test over 18 months old. Ten fish at a time were transferred from their holding tank into the neutral central section of either the background or the light preference testing tank (see Fig. 3.7) from which they could move freely into their preferred coloured compartment. This was repeated 10 times (n = 100) and the numbers of fish in each coloured compartment were recorded

every 2.5 min. over 25 minutes. Recording started 5 min after the fish were transferred. Fish have not been fed during the 30 min. period of the experiment.

The group of background–adapted fish contained fish which have been adapted to red, yellow, green, blue and white coloured tanks and the light–adapted group was adapted to these colours by coloured lighting (see Chapter 2: 2.2.2.). Fish of both groups were handled as the non – adapted group described before, with 5 repetitions (n = 50) per adaptation treatment for the background preference test (BPT) as well as for the light preference test (LPT). Background–adapted fish were also tested in the LPT and vice versa.

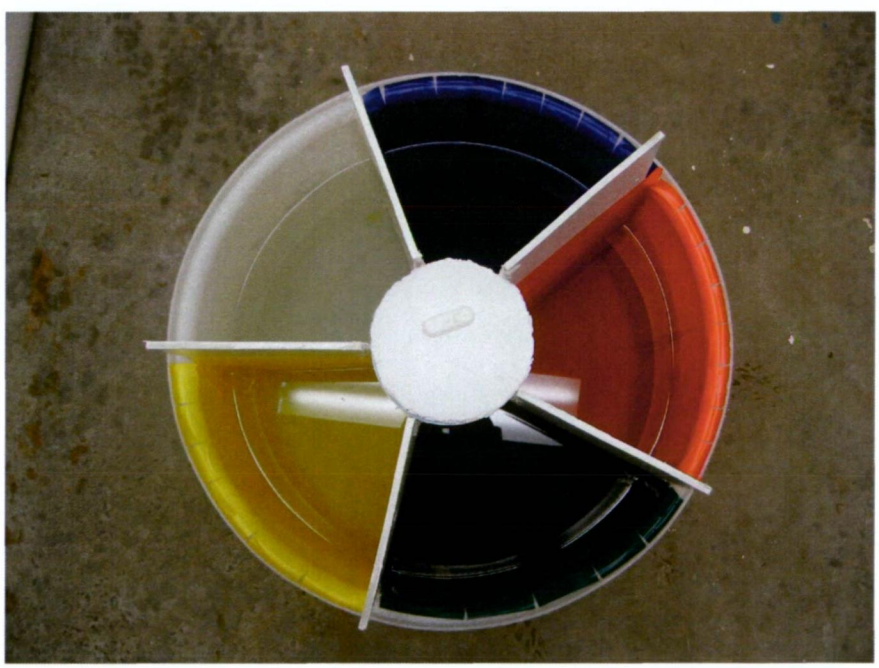

Figure 3.5: Coloured background preference testing tank from above.

Figure 3.6: Coloured light preference testing tank from above.

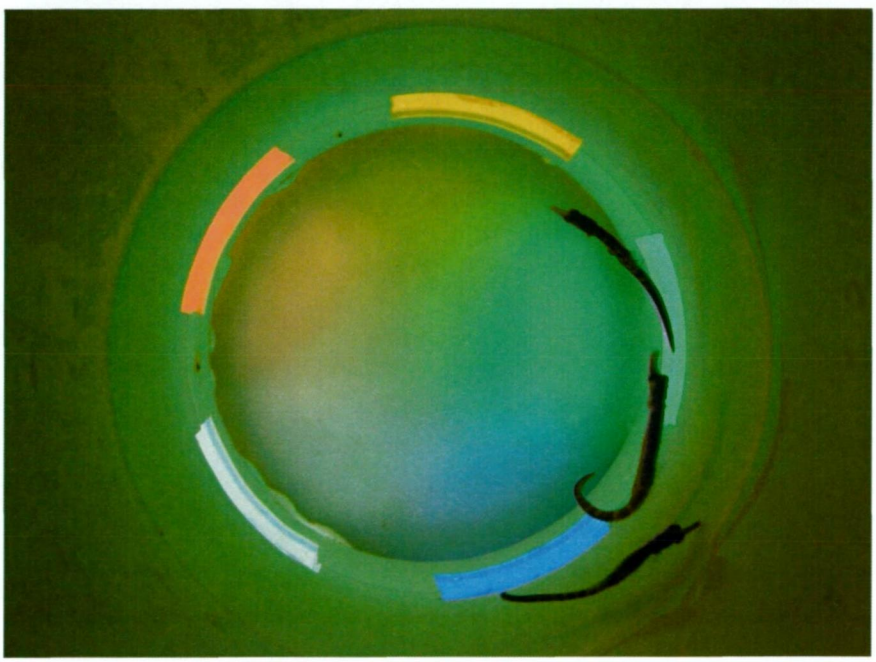

Figure 3.7: Viewing into the central compartment with openings.

3.2.4. Calculations

The raw data was standardised, where the number of fish in one compartment was divided by the total number of fish in all colour compartments. The preferences were calculated by the formula of Jacobs (1974):

$$D = (r-p)/(r+p-2rp)$$

Where = D the preference, r = frequency of use and p = frequency of the offer of the respective colour. D can range in value from -1.0 to +1.0, where negative values indicate that a colour is visited less often than the availability and therefore avoidance and positive values reflect more frequent visits and therefore a preference for a colour. The frequency of offer indicates how many choices are presented and the frequency of use is how often a certain offer was selected.

3.2.5. Statistical evaluation

All data were assessed for normality by comparing the mean preferences of ten repetitions per tank of each test colour against each other. A two–way ANOVA to compare mean preferences where replicates and treatments were considered fixed factors was used ($\alpha = 0.05$). The mean preferences for colour were also compared within different treatment colours of one system. The Tukey's HSD test was used for comparison of means if the treatment effect was significant. All statistical analyses were performed using SPSS 12.0 (2003). A graphical comparison of colour preferences for the different adaptation treatments and both preference tests was conducted for each experimental colour to determine which treatment had the most influence on the colour preferences. No statistics were run on the comparison of the different adaptation treatments.

3.3. Results

3.3.1. Results for non–adapted fish

Fish over the age of 18 months showed significantly different response to the background tank colour ($F = 39.889$ df 4, 45; $P < 0.001$). Fish of other ages were not available for the BPT and therefore could not be tested. The preferences of 18 + month old fish for the tested background colours were significantly different for red, yellow and blue against green and white (Fig. 3.8). While red (-0.94), yellow (-0.74), green (-0.18) and blue (-0.74) were disliked by the fish, white was the only preferred test colour with a mean preference of 0.67. The level of preference for white was significantly greater than green which in turn, was significantly greater than the group of red, yellow and blue.

Fish which had not been adapted to colour showed significantly different preferences for the lighting colours ($F = 1.815$, df 16, 225; $P < 0.05$). Two month old non–adapted fish (Fig. 3.9 A) avoided the test colours red (-0.52) and yellow (-0.92), while they preferred the colours green (0.20), blue (0.19) and white (0.06). Five month old fish (Fig. 3.9 B) disliked the test colours red (-0.69), yellow (-0.76) and preferred the colours blue (0.24) and white (0.27), while they showed a slight dislike (-0.03) for the test colour green. In 7 month old fish the avoidance of the test colours red (-0.93) and yellow (-1) was even stronger than in 2 and 5 month old fish. The preferences for green (0.20) and white (0.41) were positive as in 2 month old fish but 7 month old fish disliked the test colour blue (-0.41) which was significantly lower than in fish of 2 and 5 month of age. The overall preferences for fish of nine month of age (Fig. 3.9 D) were similar to the preferences of 2 month old fish for all test colours, (red (-0.71), yellow (-0.69), green (0.12), blue (0.04) and white (0.20)) with significant differentiations in the amount of the negative or positive preference value especially for red (difference 0.21), yellow (0.33) and blue (0.15). Fish over 18 months (Fig. 3.9 E) showed a similar trend

in preferences for the test colours as two (Fig. 3.9 A) and nine (D) month old fish but the values of the disliked colours were significantly lower for the test colour red (-0.73) in fish from 2, 5 and 9 month of age and significantly greater for yellow (-0.59) in fish of 2, 5, 7 and 9 month of age. The positive preference for green (0.29) was significantly greater than in fish of 2, 5, 7 and 9 month. The preference for blue (0.21) was significantly greater than in fish of 7 and 9 month of age. The white test colour (0.08) was significantly less preferred than in the fish from 5, 7, and 9 month of age. In a general sense across all groups there was a higher preference for green, blue and white compared to red and yellow lights.

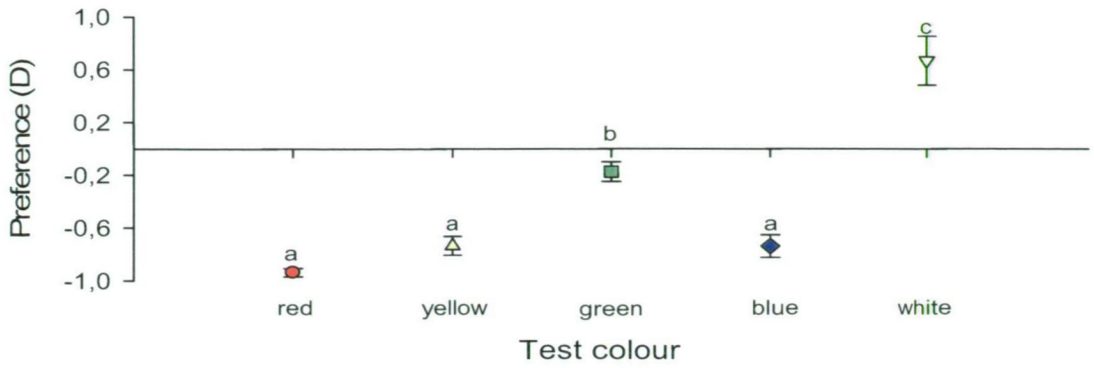

Figure 3.8: Preferences of adult seahorses of over eighteen months of age in the background colour preference test (test colours = red, yellow, green, blue, white). Superscripts indicate the level of significance tested (means ± SE).

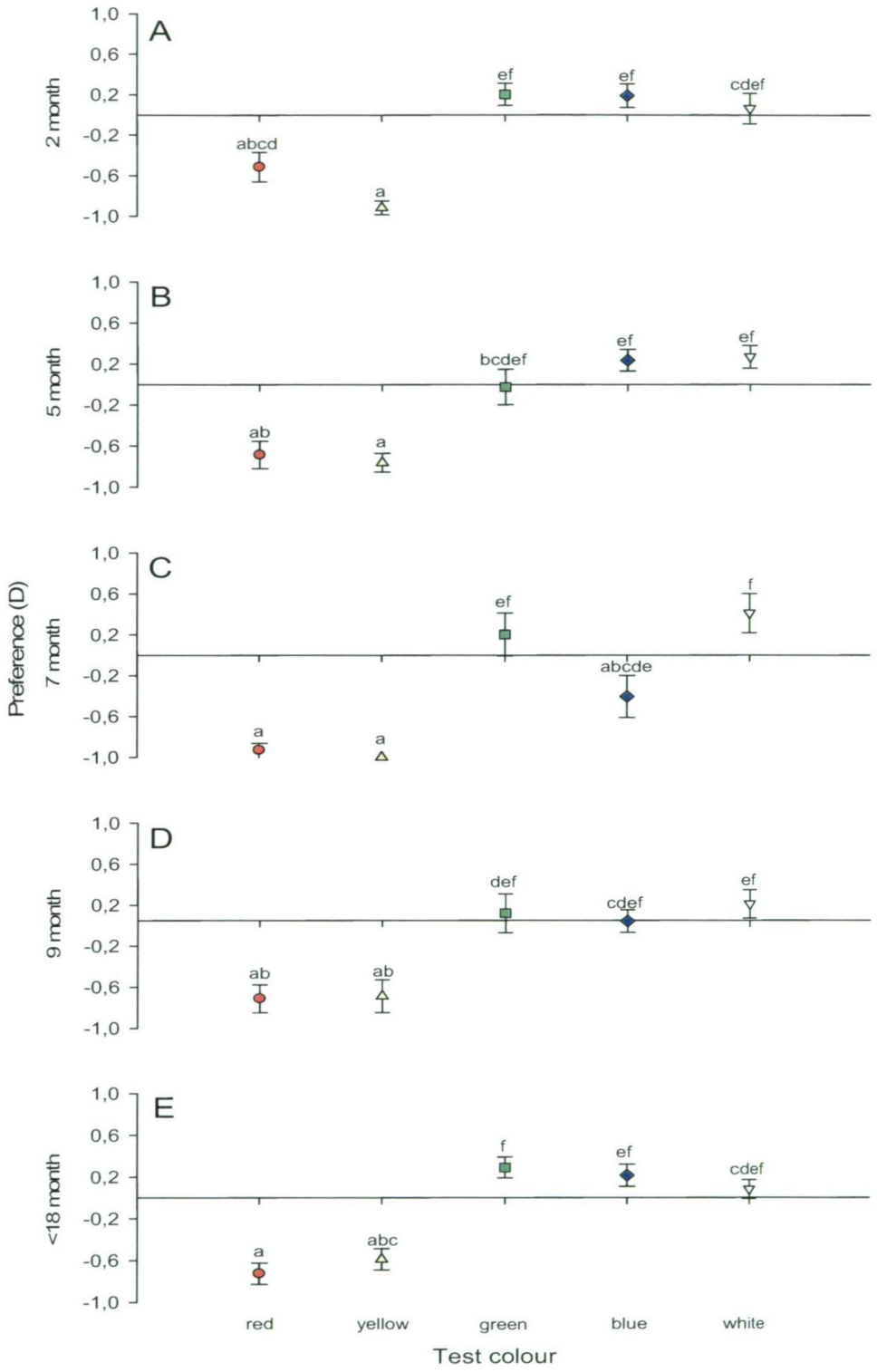

Figure 3.9: Preferences (D) of seahorses of different ages in light colour preference test (test colours = red, yellow, green, blue, white)(A): 2 month old seahorses; (B): 5 month old seahorse; (C): 7 month old seahorse; (D): 9 month old seahorse; (E): seahorse of < 18 months of age. Superscripts indicate the level of significance tested within each age category (means ± SE).

3.3.2. Results for adapted fish in the background preference–test

Colour preferences of background–adapted fish in the background preference–test

Fish which had been previously exposed to five different background colours for a period of 8 weeks (see Chapter 2: 2.2.2.) were tested to determine if the adaptation period had influenced their natural background colour preference.

Background–adapted fish did not show a clear preference for their treatment colours in the BPT ($F = 9.007$ df 24, 100; $P < 0.001$) (Fig. 3.10). Red adapted (RB) fish showed negative preferences for the test colours red (-0.24), yellow (-0.25), green (-0.30) and blue (-0.81) while white (0.47) was the only positive preferred colour (Fig. 3.10). Fish adapted to a YB completely avoided the test colours red and blue (-1). They showed a positive preference for yellow (0.23) and white (0.53) while the colour green was slightly disliked with a preference value of -0.24. The preferences for GB adapted fish were negative for red (-0.83), yellow (-0.48) and blue (-0.67) (Fig. 3.10) and the test colours green (0.11) and white (0.57) were positively preferred. Blue background adapted fish (BB) had similar preferences for the test colours as fish of RB, where the dislike of red was stronger (-0.78) and for yellow and green less (-0.12; -0.01). In WB the shown preferences were the same as RB and BB with more extreme values of negative and positive preference for all test colours and significant differences for red, yellow, green and white. In general white was preferred over the other colours.

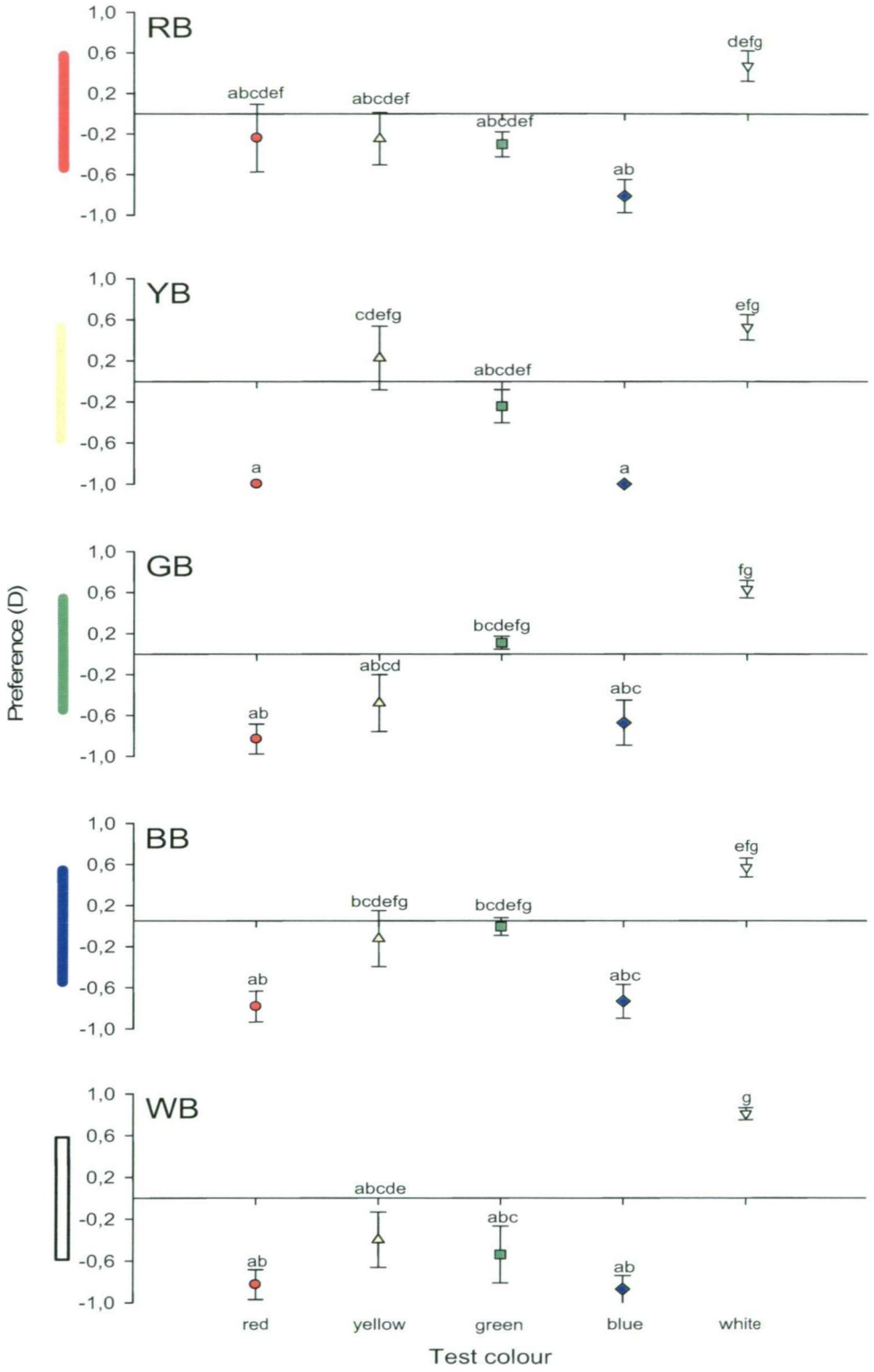

Figure 3.10: Preferences (D) for seahorse adapted to different coloured backgrounds (red, yellow, green, blue, white) over a period of eight weeks (see Chapter 2) then tested in the background colour preference test. RB = red adapted seahorse; YB = yellow adapted seahorse; GB = green adapted seahorse; BB = blue adapted seahorse; WB = white adapted seahorse. Superscripts indicate the level of significance tested across all adaptation colours (means ± SE). Where error bars are not shown, errors were too small to illustrate.

Colour preferences for light–adapted fish in the background preference–test

Fish adapted to five different lighting colours were tested as to whether the adaptation period had influenced their natural background colour preference.

The mean colour preferences for light–adapted fish in the BPT were different among the five treatments of colour ($F = 14.366$ df 24, 100; $P < 0.001$). Fish adapted to RL (Fig. 3.11) significantly preferred the white background test colour (0.48) over the other four test colours red (-0.76), yellow (-0.09), green (-0.34 and blue (-0.61). The preferences of fish from the adaptation colours YL, GL, BL and WL followed the same pattern as the one for red–adapted fish but their preference for the white background colour was greater (0.85; 0.91; 0.74; 0.77) than of RL–adapted fish. BL–adapted fish showed slight differences in their preference pattern where blue was preferred over green and the significance level for the test colour yellow (0.02) was greater than the ones of YL (-0.29), GL (-0.51) and WL (-0.19). The general pattern of background colour preference shown by these fish where white is the preferred colour is similar to background–adapted fish (Fig. 3.11).

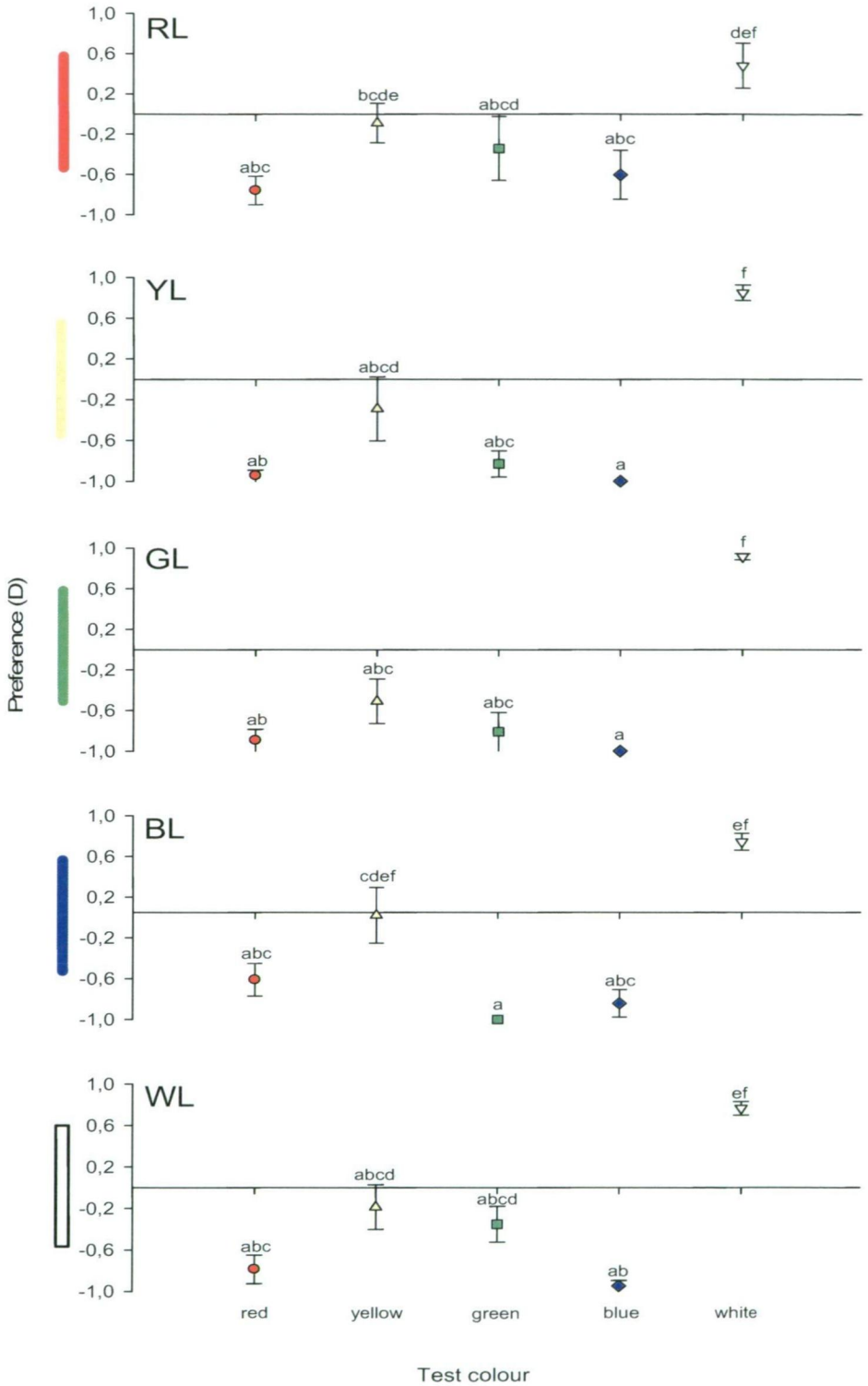

Figure 3.11: Preferences (D) for seahorse adapted to different coloured lights (red, yellow, green, blue, white) for a period of eight weeks (see Chapter 2) tested in the background colour preference test. RL = red adapted seahorse; YL = yellow adapted seahorse; GL = green adapted seahorse; BL = blue adapted seahorse; WL = white adapted seahorse. Superscripts indicate the level of significance tested through out all adaptation colours (means ± SE). Where error bars are not shown, errors were too small to illustrate.

3.3.3. Results for adapted fish in the light preference–test

Colour preferences for background–adapted fish in the light preference–test

Fish adapted to five different background colours were tested to determine whether the adaptation period (see Chapter 2) had influenced their natural light colour preference.

The mean colour preference varied significantly in fish from different coloured adaptation treatments tested in the LPT ($F = 5.41$ df 24, 100; $P < 0.001$). Colour preferences for fish adapted to RB (Fig. 3.12) in the BPT were positive for the colours yellow (0.08) and blue (0.30) whereas the colours red (-0.28), green (-0.35) and white (-0.27) were disliked by those fish. For fish adapted to YB (Fig. 3.12) the preferences were significantly different to the fish of RB for the test colours yellow (-1), which was completely avoided and for green, which was positively preferred over the other test colours (0.43). This was similar for the preferences of fish from GB (Fig. 3.12) with slight differences in significance levels for yellow, which was less avoided (-0.56) than in YB and for green, where fish showed a higher positive preference of 0.59. Fish adapted to BB (Fig. 3.12) reacted differently in the BPT than fish of the other treatments. They preferred the test colour yellow, which with a value of 0.48 was higher than RB, YB, GB and WB. BB–adapted fish avoided blue (-0.14) unlike fish adapted to RB, GB and YB which preferred blue. Adaptation to a WB (Fig. 3.12) resulted in colour preferences which were the same as in fish from RB, YB, GB and BB, for the test colours red (-0.11) and white (-0.23). For the colour blue WB adapted fish showed an avoidance (-0.23) like fish from BB.

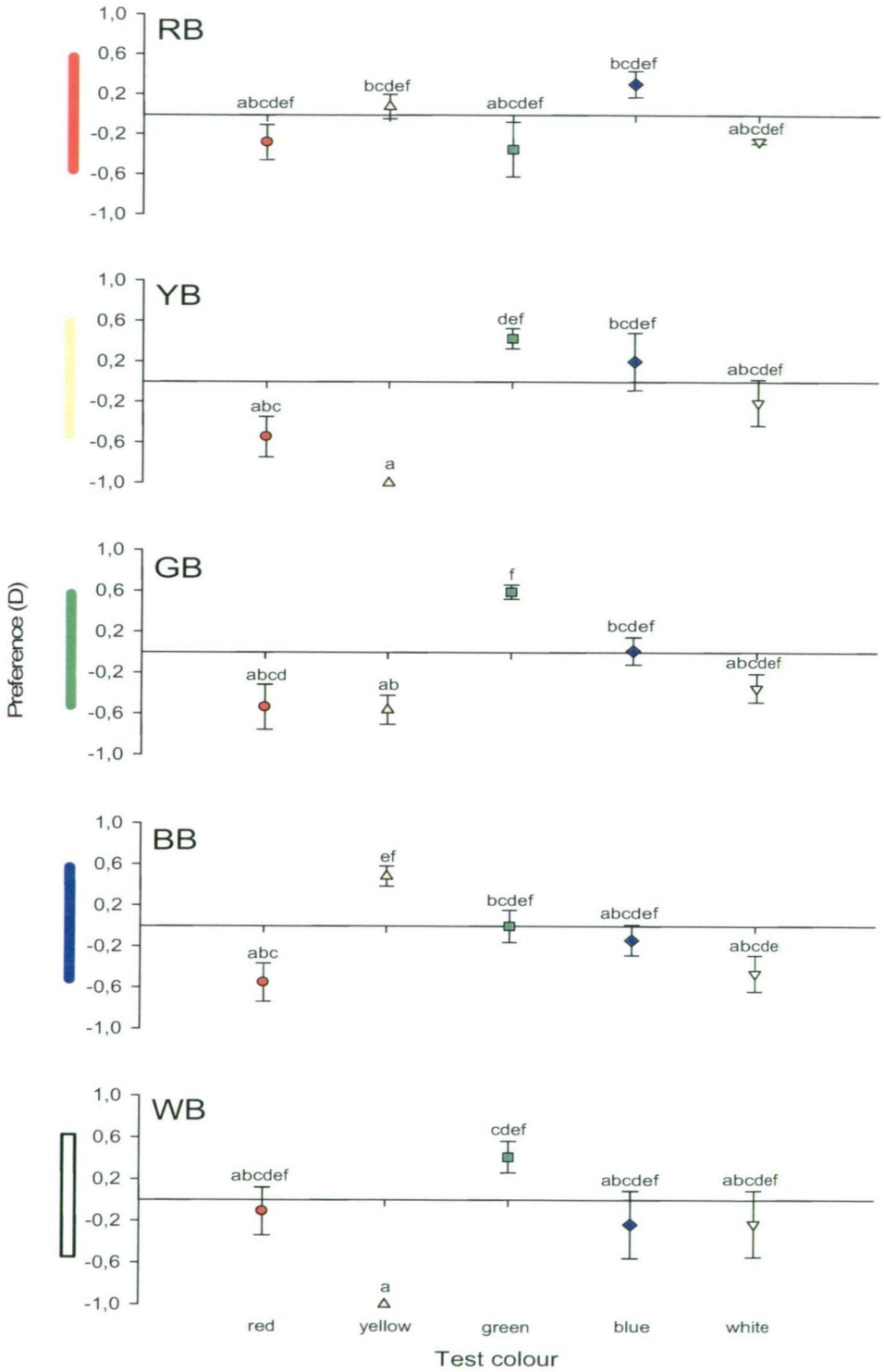

Figure 3.12: Preferences (D) for seahorse adapted to different coloured backgrounds (red, yellow, green, blue, white) for a period of eight weeks (see Chapter 2) tested in the light preference test. RB = red adapted seahorse; YB = yellow adapted seahorse; GB = green adapted seahorse; BB = blue adapted seahorse; WB = white adapted seahorse. Superscripts indicate the level of significance tested through all adaptation colours (means ± SE). Where error bars are not shown, errors were too small to illustrate.

Colour preferences for light–adapted fish in the light preference–test

Fish adapted to five different light colours were tested to determine whether the adaptation period (see Chapter 2) had influenced their natural light colour preference.

Light–adapted fish tested in the LPT showed significantly different preferences for each test colour ($F = 9.61$ df 100, 125; $P < 0.001$). Fish adapted to RL (Fig. 3.13) neither liked nor disliked the test colour red (-0.01). They showed an avoidance towards yellow (-0.29), blue (-0.29) and white (-0.19) and only positively preferred the colour green with 0.35 in the LPT. For YL adapted fish (Fig. 3.13) the preferences for colour were significantly different from RL for red (-0.63), green (0.51), blue (-0.06) and the test colour white (-0.07) while the preference for yellow was not significantly different. Colour preferences of GL–adapted fish were significantly different from other adaptation colours (Fig. 3.13 RL, YL, BL, WL) for the test colours red, yellow, blue and green. Fish completely avoided the colours red, yellow and blue (all -1) and most fish showed a positive preference for the test colour green **D** = 0.91. When comparing the fish from RL, YL, GL, BL and WL the preference for green was significantly different in fish adapted to GL. The test colour white was slightly disliked (-0.16) which was not significantly different to fish of other adaptation colours (YL, BL, WL). BL adapted fish (Fig.3.13) significantly disliked the test colours red (-0.64) and yellow (-0.79) over green (0.37) and white (0.33). The preference for blue was negative but not significantly different to the other test colours in BL. WL adapted fish (Fig. 3.13) only preferred green light (0.46) and avoided all other colours (red (-0.28), yellow (-0.42), blue (-0.16) and white (-0.65)). The avoidance for the test colour white by WL adapted fish was sig. greater than from fish of the other colours (Fig. 3.13).

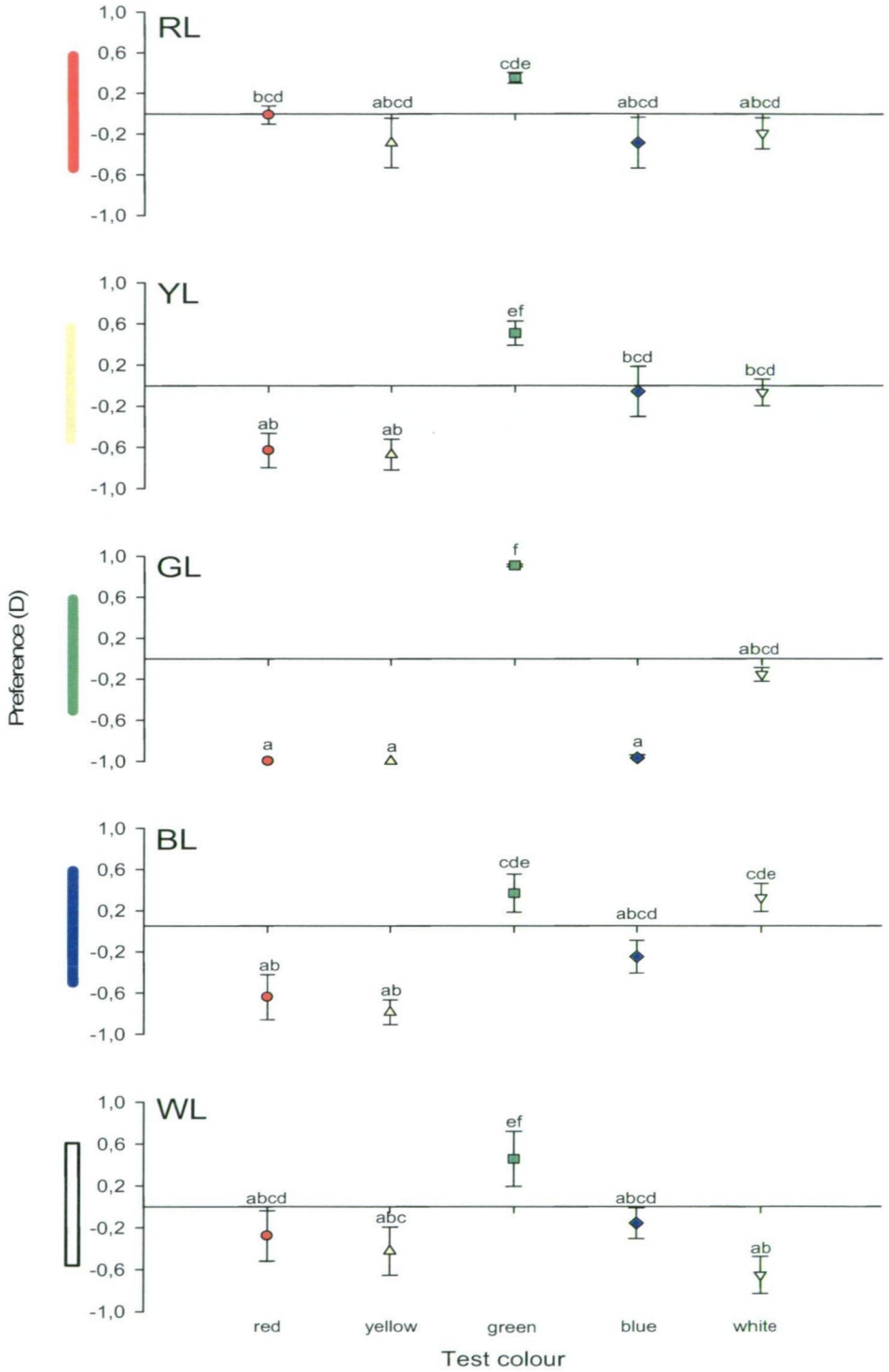

Figure 3.13: Preferences (D) for seahorse adapted to different coloured lights (red, yellow, green, blue, white) for a period of eight weeks (see Chapter 2) tested in the light preference test. RL = red adapted seahorse; YL = yellow adapted seahorse; GL = green adapted seahorse; BL = blue adapted seahorse; WL = white adapted seahorse. Superscripts indicate the level of significance. Where error bars are not shown, errors were too small to illustrate.

3.3.4. Comparison of preferences among the adaptation treatments and the two preference tests

Non–adapted fish disliked the test colour red in the BPT as well as in the LPT but adaptation of the fish to a RB and RL caused an increase in the natural colour preference for red (Fig. 3.14). RB–adapted fish avoided the test colour red less in the BPT (difference to non–adapted 0.70) than in the LPT (dif. 0.43). For RL–adapted fish there was only a difference in preference of 0.70 to non–adapted fish in the LPT.

Yellow–adapted fish tested in the BPT for the test colour yellow differed in their preferences from non adapted fish. YB adaptation influenced the preference for yellow to rise from a negative value (-0.74) of non–adapted fish to a positive value of 0.22 and for YL–adapted fish to (-0.30). In the LPT the fish did not show an increased preference for the test colour yellow.

For the test colour green non–adapted fish showed a slightly negative preference (-0.18) in the BPT and a slightly positive (0.16) in the LPT. GB adaptation increased the preference for green in the BPT (0.11) as well as in the LPT (0.59) whereas GL adaptation decreased the preference for green (-0.81) of fish tested in the BPT. Fish tested in the LPT therefore showed a positive preference for the green test colour of 0.91 which makes a difference in value of 0.75 to non–adapted fish.

Adaptation to a blue background or light did not influence the natural colour preference for blue in fish tested in the BPT (nB -0.74; BB -0.73; LB -0.84) or for those tested in the LPT (nL 0.05; BL -0.14; LL -0.25).

This was similar for the test colour white within white adapted fish in the BPT (nB 0.67; BB 0.81; LB 0.77) but adaptation had an influence on the colour preference in the LPT. Non–adapted fish had a positive preference (0.20) for the test colour white whereas WB– and WL–adapted fish disliked this colour with values of -0.23, for the background adapted fish and -0.65 for light adapted fish.

In summery non–adapted as well as adapted *H. abdominalis*, had a preference for the white background colour and the majority of non–adapted fish of different live stages

preferred the lighting colours green and white, while background and light adapted fish had a preference for the green and blue lighting colour.

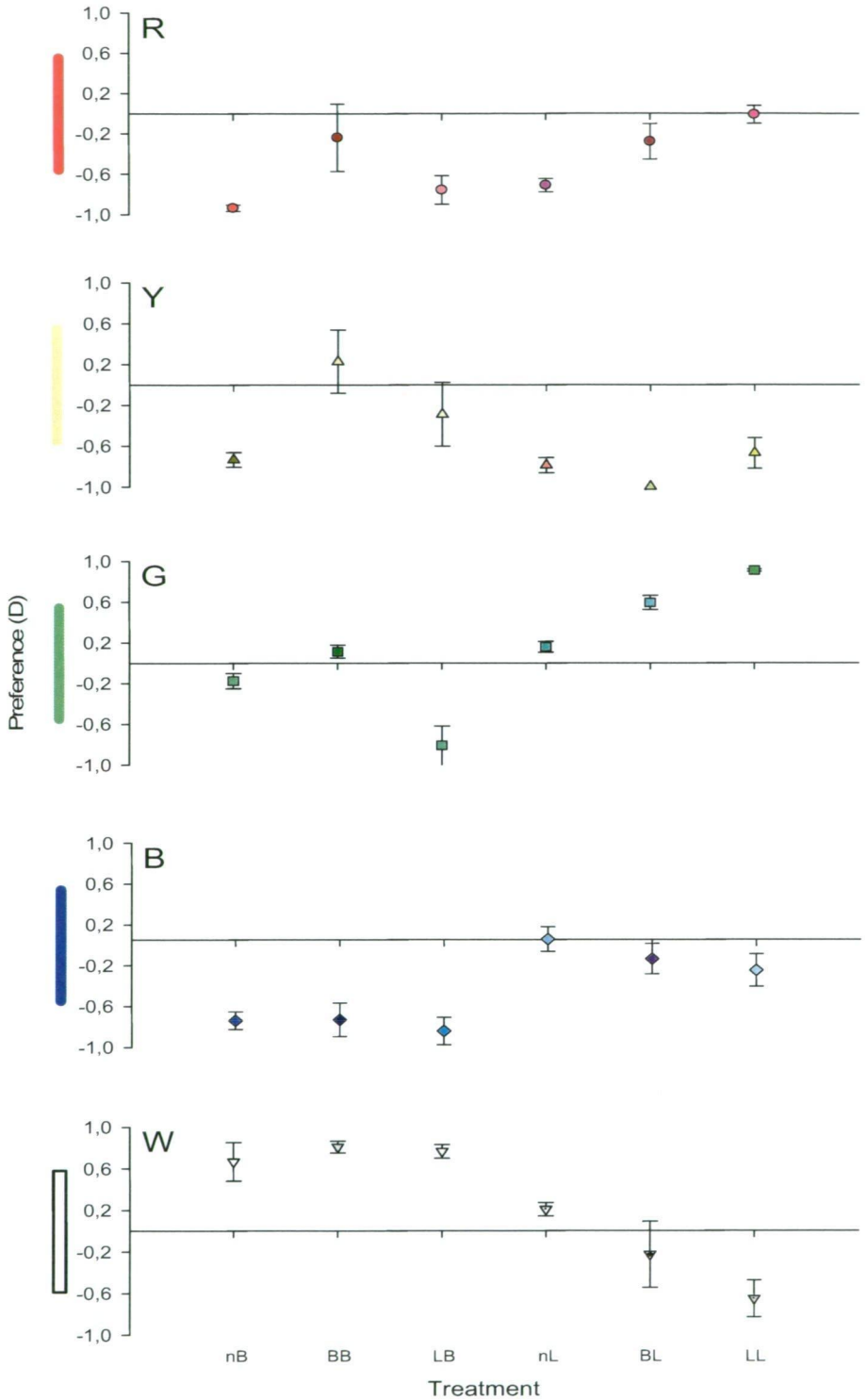

Figure 3.14: Comparison of different adaptation treatments and different tests for one test colour. nB= non–adapted in background preference test; BB= background adapted in background preference test; LB= light adapted in BPT; nL non–adapted in light preference test; BL= background adapted in LPT and LL= light adapted in LPT. Where error bars are not shown, errors were too small to illustrate.

3.4. Discussion

Colour preferences of fish were influenced by both the rearing tank colours and light colours for a period of 56 days. Fish tested prior to the adaptation period showed different preferences towards background and light colours than the fish exposed to the experimental conditions.

3.4.1. Colour preferences of fish tested in the background colour preference test

Colours play an important role in the health and behaviour of fish especially for fish cultured in aquariums or in farms. Fish are sensitive to tank and light colour and their feeding, mating behaviour and their skin colour can be influenced by the colour of the culture environment (Cunningham 1893; Douglas and Lanzing 1980; Donelly and Dill 1984; Fernandez and Bagnara 1991; Fujimoto et al. 1991; Saxena 1994; Davenport and Bradshaw 1995; Brander and McRobert 2001; Arigoni et al. 2002; Bransden et al. 2005; van der Salm et al. 2005; Yasharian et al. 2005). Understanding which colours are naturally preferred by seahorse species could help optimise holding conditions in captivity, and in turn could reduce stress and improve the survival and growth rates of seahorses produced for the ornamental trade market. This research project was undertaken to determine the colour preferences of the pot bellied seahorse (*H. abdominalis*).

In the background preference-test, non–adapted fish showed a preference for white and a dislike for the other test colours (Fig. 3.8). These results are in contrast to Kawamoto (1950; 1951), Kawamura (1996) and Muntz (1966), who found that many fish species like *Oplegnathus fasciatus,* thread-sail filefish, *Monacanthus cirrhifer,* Japanese Spanish mackerel, *Cybium niphonium, Spheroides niphobles,* Japanese barracuda, *Sphyraena japonica,* bar-faced cardinalfish, *Apogon semilineatus,* scribbled toby,

Canfhigaster rivulatus, butterflyfish, *Chaetodon auripes,* black damselfish, *Paraglyphidodon melas,* footballer, *Microcanthus strigatus,* saw tail, *Ptionurus microlepidotus,* black scraper, *Navodon modestus,* heavenly damselfish, *Pomacentrus coelestis,* Japanese horse mackerel, *Trachurus juponicus,* five-banded damselfish, *Abudefduf vaigiensis,* Goldfish, *Carassius auratus* are most attracted by green and blue backgrounds.

This could result from the fairly dark, saturated green colour of the contact paper that was used on the walls and the bottom of the test tank. The green test colour could also have been influenced by the poor reflecting surface of the contact paper (Fig. 2.5). Colour depends on the reflection and illumination spectra, and in water can depend on how an object is illuminated (Vorobyev et al. 2001). Therefore the green colour could have appeared very dark which was unattractive to fish in the background preference test. The avoidance of blue in *H. abdominalis* in the present study could be because of their natural coastal and estuarine habitat, compared to the species described by Kawamoto (1950; 1951) and Kawamura (1996), which were all pelagic species. In coastal waters and estuaries, plants and algae provide a greenish background and blue is an uncommon colour in these waters, which could also explain why the test colour green was less avoided than the test colour blue. Pot bellied seahorses prefer lighter backgrounds over dark (Woods 2000a) if given the choice, which could explain why white, was the only positive preferred test colour.

Fish adapted to the background colours red, yellow, green and white, which where tested in the background preference-test all showed a higher acceptance or a preference for the colour they were adapted too. This influence on the colour preferences (Maaß 2004) could result from a change in the eye structure induced by their new environment or on other physiological acclimation to these colours over the experimental period. Fish adapted to a blue background did not prefer their adaptation colour over the others in the test, but they avoided the colours yellow and green less. This could have been caused because fish probably perceive colours differently than humans according to evolutionary adaptation to the aquatic environment (Lythgoe 1966; 1968; 1979; Nicol 1989; Bowmaker 1990; 1995; Locket 1999; Kusmic and Gualtieri 2000). In the present

study green may have resembled blue in the perspective of fish adapted to a blue background (both are produced by short wavelength) and yellow, which has a very small spectral range 580-595 nm (Seilnacht 2006), could have been detected as green or even have been undetectable by the fish. All background adapted fish had a very clear preference for the test colour white which is the same as in non–adapted fish and can be described as natural behaviour (Woods 2000a).

Fish adapted to coloured light tested in the background colour preference test had different colour preferences than fish of non– or background–adaptation. Fish did not prefer any of their adaptation colours. All fish had a high preference for white and preferred yellow over the other test colours, although at times it was not a positive preference, but instead less avoidance. Adaptation to coloured lights seems to influence fish in different ways compared with adaptation to coloured backgrounds. Lighting colour went directly into the tanks and could be picked up by the fish's eye. In the background adaptation system whole spectrum light was used which was reflected by the tank walls and bottom before the fish could see the actual tank colour. Differences in light adapted fish may have occurred by structural changes in their cone ratio and/or spectral sensitivity to suit their new environment.

3.4.2. Colour preferences of fish tested in the light preference-test

It was found that non adapted fish of all life stages clearly preferred green, blue and white light (whole spectrum) and almost totally avoided red and yellow light in the light preference–test. A preference for green and blue seems quite natural when considering the habitat of this species. Most marine and freshwater fish prefer green and/or blue environments (Levine and MacNichol 1982). Loukaskin and Grant (1959) observed the behaviour and reactions of the Pacific sardine, *Sardinops caerulea*, under the influence of white and coloured lights and found that in a three or two colour test of different light tested against each other, green and blue were clearly preferred by around 50% over red (6.5%) and white (36%). In the two colours test the percentages of fish counted in green

or blue was nearly 100%. Kawamoto and Takeda (1950; 1951) tested six different species of young marine fish to even more light colours (8) in an almost similar test to this experiment. Their results showed that green and blue were preferred by 5 of these species. Only *Anguilla* failed to show any clear preference for one colour (Kawamoto and Takeda 1950; 1951). This resembles the findings of Maaß (unpublished data) that no colour preferences can be recorded for nocturnal fish which is probably related to an eye structure with no or few cones and therefore a poor or no colour vision. In shallow and most coastal waters the available light is restricted due to absorption and high levels of suspended particles and considerable quantities of dissolved organic material. This causes the maximum transmission to be between 460 nm and 570 nm, which lies in the blue-green region of the spectrum, depending on the purity of the water (Bowmaker 1995). The eye structure of *H. abdominalis* could be so well adapted to this environment (Levine and MacNichol 1979) that these fish naturally preferred green and blue light as tested in the light preference test. Suggestions about an approach to examine this can be found in the next chapter.

Background adaptation caused red–adapted fish to prefer yellow and blue light and to avoid the test colour red less than non–adapted fish. Yellow and blue are at the opposite sides of green in the colour spectrum, so red background adaptation could have caused a change in the visual spectrum of the fish which may have influenced the cone sensitivity from a wide range sensitivity (blue, green and red) to a reduced sensitivity for just short (blue) and long (red-yellow) wavelength with a lack for the middle range (green).

Yellow, green and white background adapted fish all had a positive preference for the test colour green and yellow adapted fish also showed a preference for blue. So these adaptation colours do not seem to influence the visual ability of the fish for lighting colours and does not change their natural preferences for green and blue light as examined in studies of Kawamoto (1950; 1951), Maaß (2004), Marchesan (2005) and Privolnev (1956). Blue background adapted fish in the present study showed a colour preference for yellow which seems to be unusual compared to the literature and leads to the idea that adaptation to the blue background may have caused changes in the colour

perception of the fish. A suggestion is that the blue background colour shifted the visual spectrum towards longer wavelength and fish may have seen the yellow test colour as green. This would have been influenced by a change in cone sensitivity.

Adaptation to different coloured lights did not cause major differences in colour preferences of fish compared to non–adapted fish. All fish showed a clear preference for the lighting colour green, which supports the findings of Kawamoto (1950; 1951), Maaß (2004; unpublished data), Marchesan (2005) and Privolnev (1956) that green is the most preferred colour of fishes. A slightly enhanced preference for red has been found for the fish adapted to red light which indicates that using red light in hatcheries could actually cause problems for the fish if they are transferred to other environments at a later date. A positive preference for white shown by blue–light adapted fish may be explained by the spectrum and intensity of the light source. White light had a higher intensity then blue light and therefore could have been attractive for the fish (Woods 2000a).

Chapter 4

Eye structure of the pot bellied seahorse

H. abdominalis

4.1. Introduction

4.1.1. The fish eye

The basic components of the eye in fishes do not differ from those of other higher vertebrates (Fig. 4.1). However, fishes have an enormous variety of different eye sizes, structures and positions between species. These specialisations allow fish to obtain sufficient information from diverse and unique (under water) light environments (Koubara and Yamada 1995).

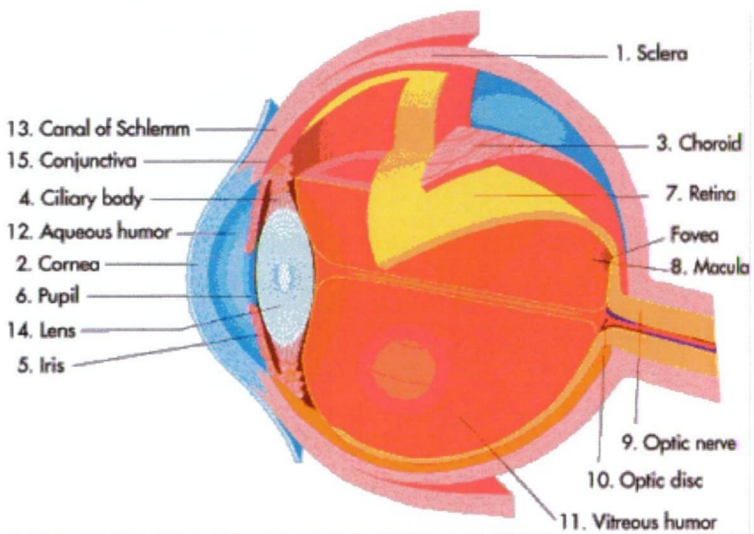

Figure 4.1: Vertebrate eye demonstrating all basic components (University of Canberra 2007).

The eyes of fishes are generally ellipsoid, and are composed of three concentric layers: an external layer that consists of the sclera and the cornea, a middle or vascular layer consisting of the choroids, ciliary body and iris, and an inner layer of nerve tissue, the retina (Douglas and Djamgoz 1990; Junqueira et al. 1995; Koubara and Yamada 1995; Kessel 1998). Monocular vision is possible where eyes are located laterally on either side of the fish's body.

The lens is situated at the front of the eye, and has a large radius for incoming light. This allows a very large field of vision with angles of 166°-170° horizontally and 150° vertically (Suworow 1959). The cornea provides protection in front of the lens and the vitreous space behind the lens surrounds the retina. The retina contains the photoreceptor cells and the sensory epithelium which receives light stimuli and transfers data to the brain via the optic nerve (Douglas and Djamgoz 1990; Junqueira et al. 1995; Koubara and Yamada 1995; Kessel 1998).

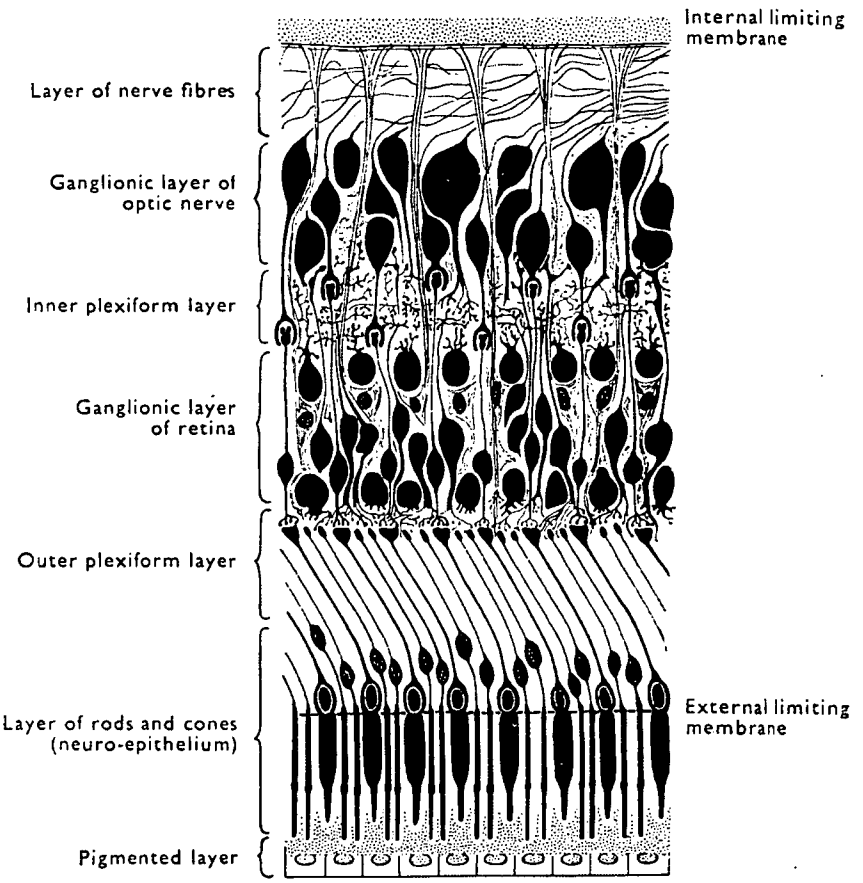

Figure 4.2: Schematic drawing of the layers of the retina (Cunningham 1981).

4.1.2. Retinal layers

The retina is made up of a number of different layers which, from the innermost to the outermost, are as follows: ganglion cell layer (nerve fibres and ganglionic layer of optic nerve), inner plexiform layer, inner nuclear layer (ganglionic layer of retina), outer plexiform layer, outer nuclear layer and photoreceptor layer (layer of rods and cone cells) and the pigment epithelium (Fig. 4.2). Although the fundamental structure of the retina is similar in most fish, histological comparisons among species give some important information which indicate specific adaptive strategies for their particular light environment (Koubara and Yamada 1995). Differences can occur in layer thicknesses, sagittal mosaic cell formations and the relative number of rods and cones in the same area of the retina (Douglas and Djamgoz 1990).

Rods and cones are two different kinds of photoreceptor cells existing in the outer layers of the retina. Rods allow black and white vision in dim light and cones allow colour vision and visual acuity in higher light intensity (Nicol 1989; Bowmaker 1990; Douglas and Djamgoz 1990; Junqueira et al. 1995; Kessel 1998). Rods are long cells with cylindrical outer segments (Kessel 1998) and elongated myoids, and usually terminate in spherules. Cones typically have short, stout, conical outer segments (Kessel 1998), large bulbous ellipsoids, and stout myoids and terminate in pedicles. Recognition of the two categories of photoreceptors led to formulation of the duplex theory of vision, according to which the rods participate in scotopic (night) and the cones in photopic (day) vision (Nicol 1989; Bowmaker 1990; Douglas and Djamgoz 1990).

The outer segments are modified, closely–spaced cilia which contain membrane–limited saccules which lie parallel to each other and usually perpendicular to the long axis of the segment. The visual pigments lie in the sac membrane in a lipoprotein matrix, and the plane of the chromophore (light sensitive pigment) is parallel to the surface; this accords with the physiological condition whereby light is absorbed parallel to the plane of the electric vector (Nicol 1989; Junqueira et al. 1995; Kessel 1998; McKenzie and Klein 2000). The outer segment joins the inner segment by a narrow eccentric connecting stalk of the modified cilium (Nicol 1989; Koubara and Yamada 1995;

Kessel 1998; McKenzie and Klein 2000). Microtubules of the cilium extend into the cytoplasm bordering the rod outer segment and into the accessory outer segment of the cone (Nicol 1989).

4.1.3. Cones and mosaics

Cones can contain different visual pigments, of the opsin family, and have different structures depending on the environmental conditions a species live in (Nicol 1989). They can be differentiated in single, double, twin (identical or non–identical) (Losey et al. 2003), triple and even quadruple cones (Bowmaker 1995). Single cones are mostly sensitive for short wavelength (SWS) (UV, blue) and double and twin cones for middle wavelength (MWS) (green) and/or long wavelength (LWS) (red) (see Fig. 4.2). The closer a species lives to the ocean surface the higher the likelihood to have more than two classes of cones (Loew and Lythgoe 1978; Levine and MacNichol 1979) because the spectral range of light near the surface is broader than in deeper waters. The cones are not randomly distributed in the retina but follow a highly organised pattern of rows and squares of which the cross section is called a mosaic (Bowmaker 1995). It is possible to infer which cells in a mosaic contain specific visual pigments, since certain pigments are present in particular cone types which are found in particular positions in the retinal mosaic (Bowmaker 1995) (Fig. 4.3).

It is known that the light spectrum and intensity have a demonstrated effect on retinal morphology in fish (Nicol 1989; Bowmaker 1990; 1995; Reckel et al. 2002). In aquaculture, light spectra and background colour can affect feeding, distribution of fish in the tanks and behaviour (Corazza and Nickum 1981; Harder and Summerfelt 1996; Tamazouzt et al. 2000; Jentoft et al. 2006; Cobcroft unpublished). In this study the light spectrum and background colour were manipulated to investigate effects on skin colour of *H. abdominalis* (Chapter 2) but it is also important to understand possible affects on retinal morphology/ structure to ensure detrimental affects are avoided. So far it is

known that syngnathid retinas have single and double cones which are arranged in regular square units, that rods are very sparsely present and that a fovea is regularly present in syngnathid retinas (Engström 1963; Wagner 1971; Ali and Anctil 1976; Mosk 2004). The presence of rods can be estimated by calculating the ratio of cone ellipsoids to photoreceptor nuclei (Blaxter and Jones 1967), because in light–adapted fish rod nuclei are visible and countable in the outer nuclear layer but do not have an ellipsoid.

In this study, the retinal morphology of the colour adapted fish (from Chapter 2) was examined, focussing on photoreceptor density, retinal layer thickness and mosaics, to describe the eye of *H. abdominalis* and changes to the retina in relation to the background colour and light environment during the adaptation time.

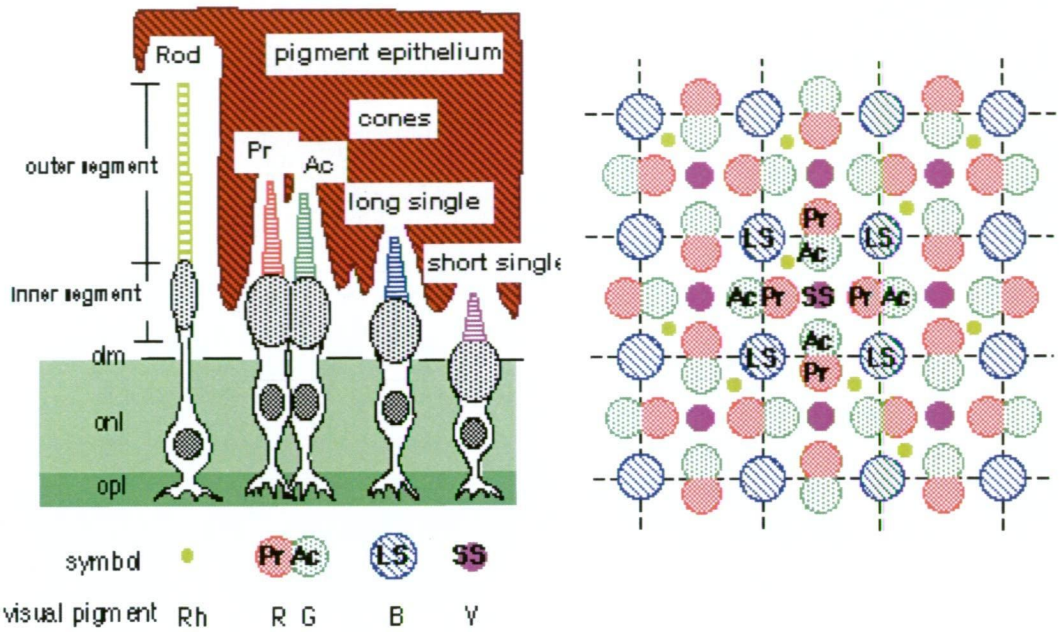

Figure 4.3: Different cones and their spectral sensitivity (left) and retinal mosaic (right) (Hisatomi et al. 1997). R = red, G = green, B = blue, V = uv, Pr = preliminary double cone, Ac = accessory double cone.

4.2 Materials and Methods

4.2.1. Experimental fish

Fish used were all sampled during the day, at the light period, and from a 56 day long colour adaptation experiment with the colours red, yellow, green, blue and white (see Chapter 2: 2.2.1 – 2.2.3 for details)

4.2.2. Experimental systems

The system used was identical to that in Chapter 2: 2.2.2. Light intensities measured during the experiment ranged between 17–145 lx (Chapter 2: 2.3.1 Tables 2.2 and 2.4.)

4.2.3. Experimental protocol

See Chapter 2: 2.2.3.

4.2.4. Histology Sampling

Retinal samples were taken from fish for structural analysis of cone mosaics, layer thicknesses and cone counts following the adaptation experiment described in Chapter 2. Three fish of visibly different hues were selected from every tank (3 fish/ tank x 5 treatments x 3 or 4 replicates). Hues were categorised as bright, medium and dark skin colour for each tank. The fish were taken from their tanks and euthanased by anaesthetic over doses (Benzocaine, 500 mg l^{-1} water). Their heads were fixed in 5% glutaraldehyde in 0.1 M sucrose-phosphate-buffer (2 g sucrose 100 ml^{-1}) of pH 7.4 at 4°C for 24 h (Cobcroft and Pankhurst 2003). Samples were then rinsed 3 times in sucrose-phosphate-

buffer to remove the fixative and transferred to 70% ethanol. The ethanol was changed once after a week and the samples stored until processed.

4.2.5. Embedding

The samples (all from the medium hue category) were dehydrated in an ethanol series of 80% and 2 x 100% and the heads were dissected dorsally to separate left and right eyes. Tissues were placed in a mould and embedded in glycol methacrylate resin (JB4 resin, ProSciTech, Australia). All left eyes were sectioned in the transverse plane and right eyes in the sagittal plane, using glass knives, to 3 μm thickness. Ten sections were placed on each slide and left to dry at room temperature for 30 min before staining the sections with a polychrome stain (Wikeley and Goodsell 1994) and mounting them with Shur/mount™ (ProSciTech, Australia) for light microscopy (Cobcroft and Pankhurst 2003). It was not possible to section some samples because the resin was too soft and therefore new samples from the bright category were embedded and then sectioned following the same protocol. Samples were labelled individually to identify from which category they came from.

4.2.6. Measurements and cell counts

The transverse sections of all samples were photographed under 32 x magnification using an Olympus BH 2–RFCA microscope with attached camera (Leica DC 300 F) and image capturing software (Leica IM 50). The eye diameters were measured using ImageTool 3.00. The section with the widest diameter (ventral–dorsal) from each sample was recorded and further pictures of this section taken under 625 x magnification. Measurements were made in 3 regions of the eyes (dorsal, medial and ventral) (see Appendix D: Fig. D 1) in which cone, nuclei counts and layer thickness measurements were made in 3 transects of 50 μm. Three of the samples (LG 3, BG 4 and BW 4) were excluded because parts of the retina were missing. Samples from the

middle and bright skin colour categories were pooled after the first examination did not show any differences in retinal structure between both categories.

The pigment index was calculated with the formula:

$$\mathbf{pi = p/v}$$

pi = pigment index, which can have a value of 0 – 1, p = thickness of pigment epithelium (μm), v = thickness of visual cell layer or light path length (PE+OS+EI; PE = pigment epithelium, OS = outer segments, EI = ellipsoids) (μm) (Ali 1961; Blaxter and Jones 1967; Blaxter and Staines 1970; Masuma et al. 2001) to compare the amount of pigment migration and visual adaptation to the different treatments (Ali, 1961; Blaxter and Jones, 1967). The light path length (PE+OS+EI) is the distance available for light capture, which is related to the relative sensitivity/ capacity to capture available light. All retinal layer measurements were absolute values (μm) and then calculated relative to the width of the whole retina at each replicate transect to obtain the relative layer thickness.

4.2.7. Statistical analysis

The data was assessed for normal distribution by examining residual plots. Eye diameters and fish weight were correlated to test independence of data. A 2–way ANOVA was performed to analyse data for interactions between treatment colours and eye positions for differences in layer thickness. If no significant interactions were found, a 1–way ANOVA was used to analyse data for differences between treatment colours in each system (background, light) and between retinal position (dorsal, medial and ventral). The cone ellipsoids and photoreceptor nuclei counts were compared with a ratio for the detection of rods; number nuclei: number cone ellipsoids, where the ratio is > 1: 1 it indicates that rods are present.

4.3. Results

4.3.1. Background adapted fish

Cones and nuclei density

There were a similar number of cone ellipsoids and photoreceptor nuclei in all fish, indicating very few rods were present, so nuclei counts were not analysed further and were used as a control for the cone counts. Cones had significantly higher densities in the medial position (14.28) compared to the dorsal (12.70) and ventral (12.09) positions ($F = 24.43$; df 2, 161; $P = 0.000$). Colour had no significant influence on cone densities at the dorsal ($F = 0.48$; df 4, 53; $P = 0.747$) and ventral ($F = 1.30$; df 4, 53; $P = 0.284$) positions but significant differences were found between the adaptation colours in the medial position ($F = 2.70$; df 4, 53; $P = 0.041$). Densities of cones were significantly lower in RB (13.50) adapted fish than in YB (15.50) adapted fish (Figure 4.4).

Light path length

The relative thickness of the pigment epithelium (PE) was significantly different in background–adapted fish with an interaction between treatment colours and positions ($F = 2.72$; df 14, 161; $P = 0.008$) (Table 4.1). In the dorsal position of the retina no significant differences in the thickness of PE was found between the 5 adaptation colours. In the medial position, GB–adapted fish had a significantly thinner relative PE (11 ± 1 %) than RB–adapted fish (16 ± 1) and in the ventral position WB–adapted fish had a significantly thicker relative PE (21 ± 1 %) compared to fish from RB (16 ± 2 %) (Fig. 4.5).

The absolute thickness of the PE plus the outer segments (OS) and ellipsoids (EI) (PE+OS+EI) was significantly different with an interaction of treatment colour and eye

position (F = 3.48; df 14, 161; P = 0.001). The absolute thickness of the PE+OS+EI in the dorsal position of GB–adapted fish was significantly greater (748 ± 60 μm) than in YB– (514 ± 27 μm) and BB–adapted (382 ± 29 μm) fish. In the medial position no significant differences in the absolute thickness of PE+OS+EI was found among the five adaptation colours. In the ventral position GB–adapted fish had a significantly thicker absolute PE+OS+EI (756 ± 37 μm) than fish from RB (548 ± 29 μm), YB (452 ± 41 μm) and BB (505 ± 52 μm) (Fig. 4.6 and Table E 1).

The pigment index (pi) for background adapted fish was not significantly different between the 5 colour treatments (F = 0.73; df 4, 161; P = 0.573) (mean = 0.54) but showed a significant difference for the eye positions (F = 18.10; df 2,161; P = 0.000). The pi was lowest in the ventral region of the eye (0.47) and highest in the medial eye position (0.60) (see Fig. 4. 7).

Thickness of retinal layers

Absolute retinal thickness was larger in the medial region (2976.44 μm) for all treatments compared with the dorsal (1821.05 μm) and ventral regions (1615.22 μm) (F = 66.70; df 2, 161; P = 0.000). For all colours the dorsal retinal thickness was higher than the ventral except for blue (Fig. 4.8). GB adapted fish had the thickest retina in all 3 positions of the eye (Table E 1, Fig. 4.8, Fig. 4.14).

The relative thicknesses of the retinal layers (Table 4.1) were similar between the treatments with a few exceptions in relative thickness of PE and the ganglion cell layer (GC). GB– and BB–adapted fish had the smallest PE in the dorsal position (15 ± 1 %) compared to RB– (19 ± 2 %), YB– (18 ± 1 %) and WB– (17 ± 0 %) adapted fish. GB–adapted fish also had the smallest relative PE in the medial position (11 ± 1 %) as well as a thicker GC (16 ± 1 %) than RB– (10 ± 1 %), YB– (10 ± 1 %) and BB– (13 ± 2 %) adapted fish. In the ventral position WB–adapted fish had the significantly thickest relative PE (21 ± 1 %) (Fig. 4.8).

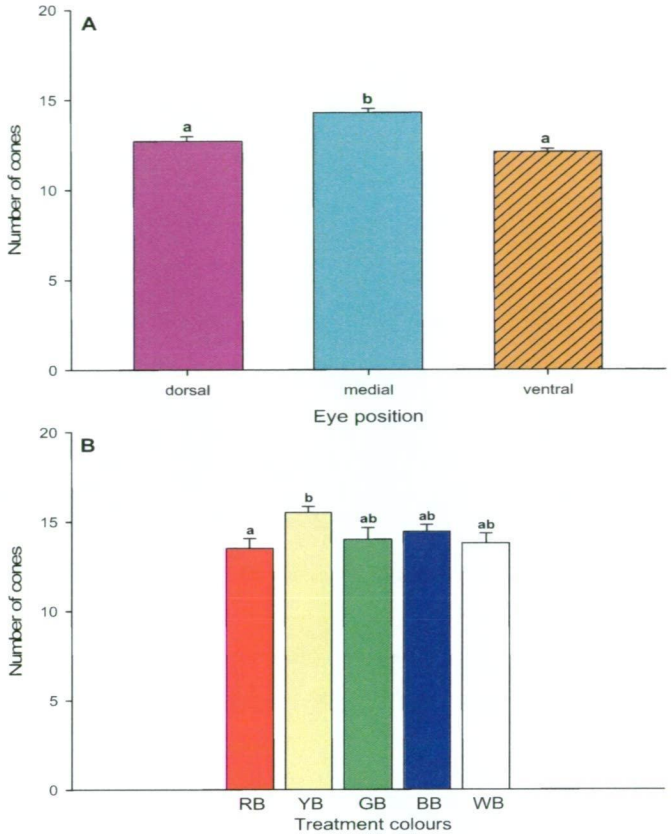

Figure 4.4: Number of cones of background (red, yellow, green, blue, white) adapted *H. abdominalis* in relation to A: position in the eye and B: tank colours for the medial eye position.

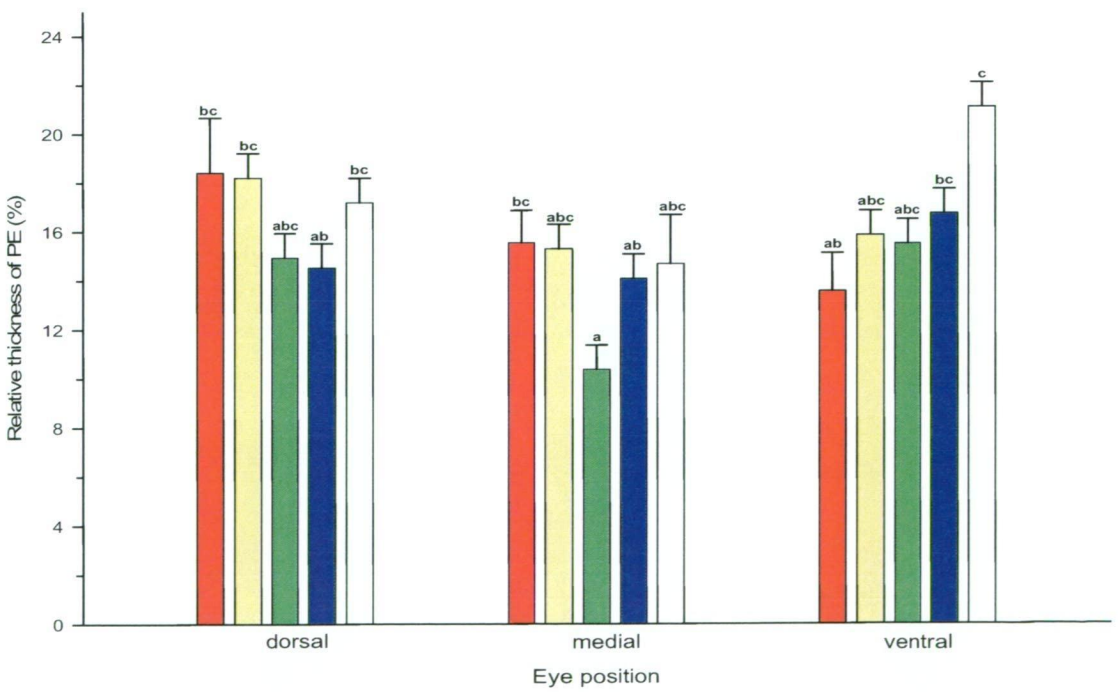

Figure 4.5: Relative thickness of the pigment epithelium for the background (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days.

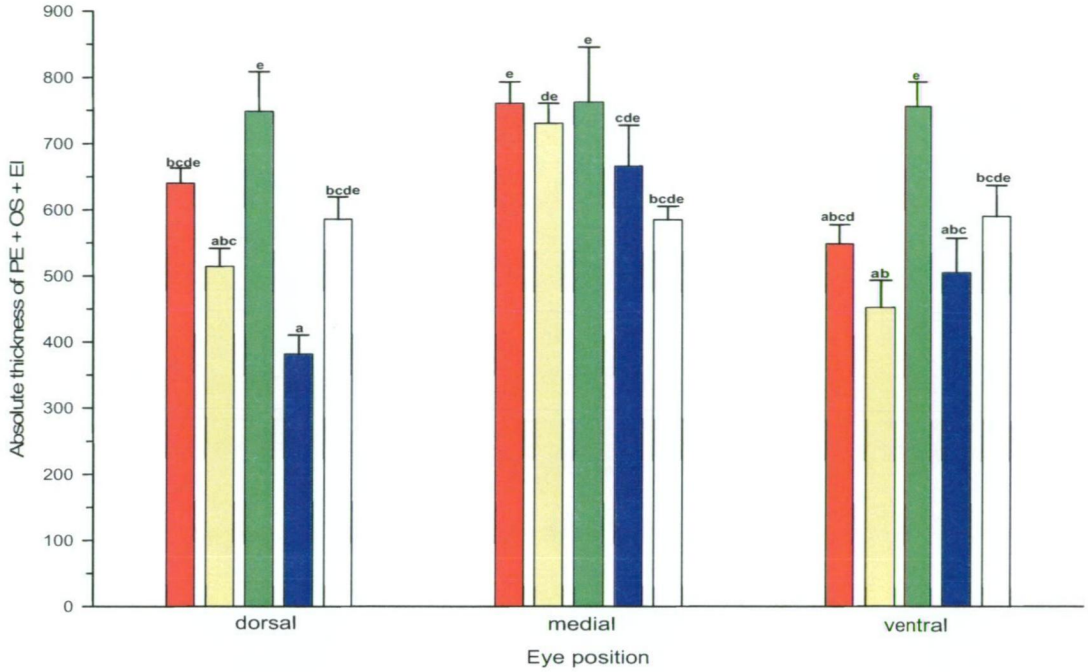

Figure 4.6: Absolute thickness of the light path length (pigment epithelium, outer segments and the ellipsoids) through the retina for the background (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days.

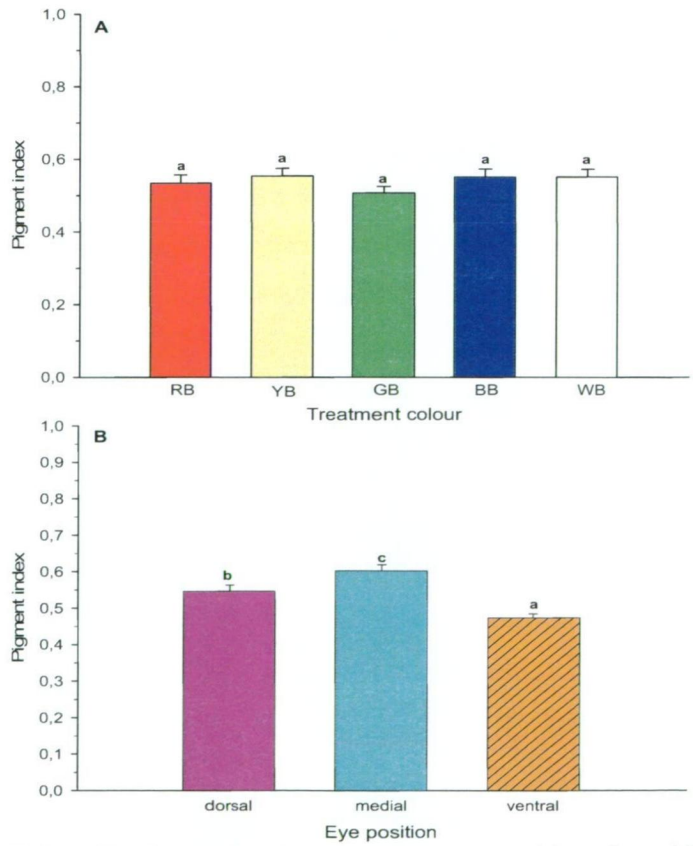

Figure 4.7: Pigment index of background (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days, in relation to A: background tank colour and B: position in the eye.

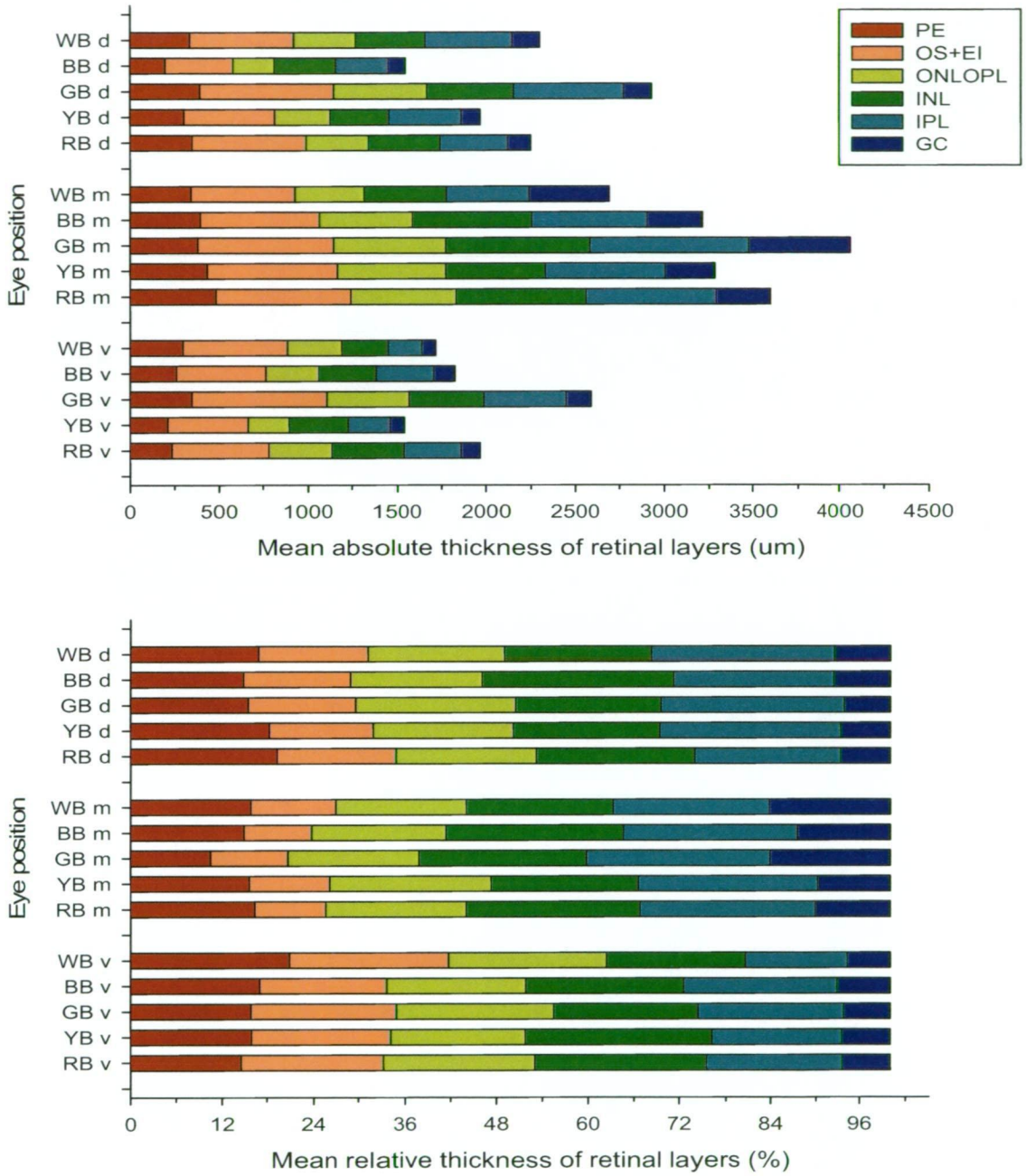

Figure 4.8: Mean absolute and relative layer thicknesses of background (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days (d = dorsal; m = medial; v = ventral).

4.3.2. Light adapted fish

Cones and nuclei

In light adapted fish cone densities were significantly higher in the medial section of the eye (13.90) compared to the dorsal (12.57) and ventral (12.50) position ($F = 8.59$; df 2, 125; $P = 0.000$). Colour had no significant influence on the cone densities of light adapted fish in all three retinal sections of the eye. The cone densities of RL (11.56) and BL (11.67) adapted fish were lower than in the other three adaptation colours (mean = 13.24), although the difference was not significant ($F = 0.99$; df 4, 125; $P = 0.416$). Numbers of the nuclei were similar to the cones ellipsoids (ratio approx = 1) so no further analyses were applied and nuclei counts were handled as a control for the cone counts (Figure 4.9).

Light path length

In light adapted fish no interactions were found between the relative thickness of PE and treatment colours or regions in the retina ($F = 1.33$; df 14, 125; $P = 0.238$) and both factors were therefore analysed separately. GL adapted fish had the thinnest and significantly lower relative PE (11.60 %) than YL (16.33 %), BL (16.28 %) and WB (16.02 %) ($F = 4.14$; df 4, 125; $P = 0.004$). The relative PE in the dorsal section (13.29 %) of the eye was significantly thinner than in the medial (15.62 %) and ventral sections (16.62 %) ($F = 6.03$; df 2, 125; $P = 0.003$) (Fig. 4.10).

There were also no interactions found between the absolute thickness of PE+OS+EI and treatment colours or eye position ($F = 0.74$; df 14, 125; $P = 0.660$). RL (597.44 µm) and WL (584.59 µm) adapted fish had the significantly thickest PE+OS+EI and YL (504.13 µm) adapted fish had a thicker absolute PE+OS+EI than BL (418.04 µm) adapted fish ($F = 11.83$; df 4, 125; $P = 0.000$). Absolute thickness for PE+OS+EI of GL (548.85 µm) adapted fish was significantly greater than in BL adapted fish. The dorsal eye position

of the retina was significantly thinner (445.99 µm) than medial (632.74 µm) and ventral (509.21 µm), which in turn was significantly thinner than medial ($F = 37.10$; df 2, 125; $P = 0.000$) (Fig. 4.11, Table E 2).

The pi for fish adapted to different lighting colours ($F = 3.103$; df 14, 125; $P = 0.018$) was significantly smaller in GL (0.45) adapted fish than for fish of the other four colours (mean = 0.56) and a significantly greater pi ($F = 27.88$; df 2, 125; $P = 0.000$) was calculated for the medial part (0.64) of the eye (Fig. 4.12).

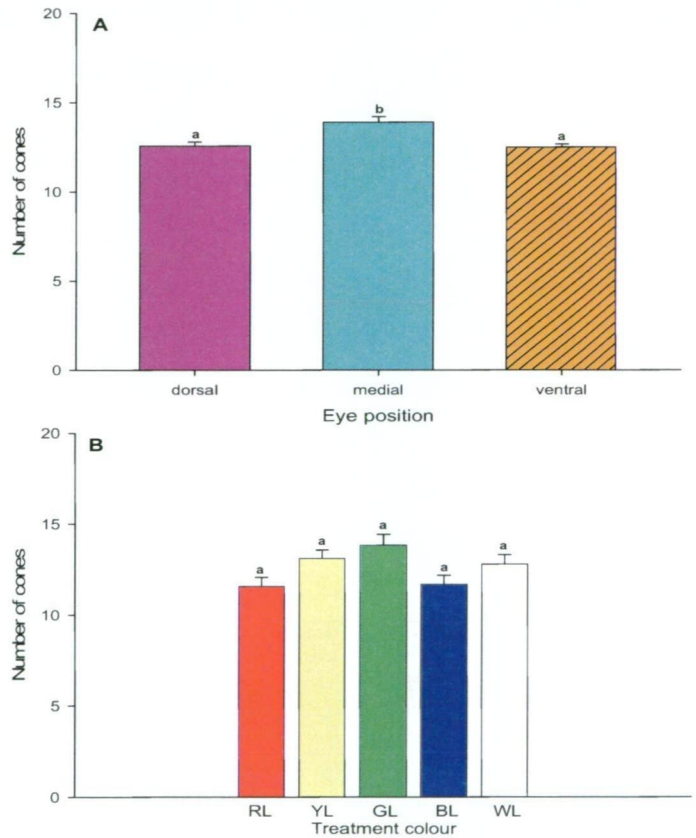

Figure 4.9: Number of cones of light (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days in relation to A: position in the eye and B: lighting colours for the ventral position of the eye.

Thickness of retinal layers

The absolute retina was thicker in the medial region (mean = 2703.90 μm) than in the dorsal (mean = 1798.83 μm) and ventral regions (mean = 1493.95 μm) for all colour treatments ($F = 45.08$; df 2, 125; $P = 0.000$). Fish from RL, YL and BL tanks had a thinner dorsal retina (compared to their ventral retina thickness) and GL and WL adapted fish had a thicker dorsal than ventral retina.

The relative thickness of the retinal layers (Table 4.2) was similar in most fish except for the thickness of relative PE in GL adapted fish (v = 13 ± 2 %, m = 13 ± 3 %, d = 8 ± 3 %), which was thinner than for other fish in all three positions of the eye (mean: v = 17.25 ± 2.75 %), a larger GC layer in the medial part of WL (22 ± 8 %) and the ventral part of BL adapted fish (15 ± 8 %) and a smaller GC layer in YL adapted fish in the medial position (12 ±1 %) (Fig. 4.13, Table E 2).

4.3.3. Mosaics

The cone mosaics in all colour treatments were very similar except for blue treatments. A square mosaic with four double cones around one single cone was found in background and light adapted fish (Fig. 4.15 – 4.17) however fish exposed to blue lighting or backgrounds had some rows with two or even three single cones in the middle of the 4 double cones (Fig. 4.17).

Table 4.1: Relative thickness (means ± SE) of retinal layers for all adaptation colours and eye positions of background adapted seahorse. F and P (df 14, 161); 2 – way ANOVAS (treatment colour/ eye position).

Background adaptation colour		Red	Yellow	Green	Blue	White	F	P
Relative thickness of retinal layers (%) (mean + SE)								
PE	**dorsal**	19 ± 2	18 ± 1	15 ± 1	15 ± 1	17 ± 0		
	medial	16 ± 1	16 ± 1	11 ± 1	15 ± 1	16 ± 1		
	ventral	16 ± 2	16 ± 1	16 ± 1	17 ± 1	21 ± 1	2.72	0.008
OS + EI	**dorsal**	15 ± 1	14 ± 2	14 ± 2	14 ± 1	14 ± 0		
	medial	9 ± 1	10 ± 1	10 ± 1	9 ± 1	11 ± 1		
	ventral	19 ± 1	18 ± 1	19 ± 2	17 ± 1	21 ± 1	0.47	0.877
ONL + OPL	**dorsal**	18 ± 1	18 ± 1	21 ± 1	17 ± 1	18 ± 0		
	medial	18 ± 1	21 ± 1	17 ± 1	18 ± 1	17 ± 1		
	ventral	20 ± 1	18 ± 1	21 ± 1	18 ± 1	21 ± 1	3.49	0.001
INL	**dorsal**	21 ± 1	19 ± 1	19 ± 0	25 ± 1	19 ± 0		
	medial	23 ± 1	19 ± 1	22 ± 1	23 ± 1	19 ± 1		
	ventral	23 ± 1	24 ± 2	19 ± 1	21 ± 1	18 ± 1	3.72	0.001
IPL	**dorsal**	19 ± 1	24 ± 1	24 ± 1	21 ± 1	24 ± 0		
	medial	23 ± 1	24 ± 1	24 ± 2	23 ± 1	21 ± 1		
	ventral	18 ± 1	17 ± 1	20 ± 2	20 ± 1	14 ± 1	3.09	0.003
GC	**dorsal**	6 ± 1	6 ± 0	6 ± 0	7 ± 1	7 ± 0		
	medial	10 ± 1	10 ± 1	16 ± 1	13 ± 2	16 ± 3		
	ventral	6 ± 0	6 ± 1	6 ± 0	7 ± 1	6 ± 0	2.45	0.016

PE = Pigment epithelium; OS = outer segments; EI = Ellipsoids; ONL = outer nuclei layer; OPL = outer plexiform layer; INL = inner nuclei layer; IPL = inner plexiform layer; GC = ganglion cells.

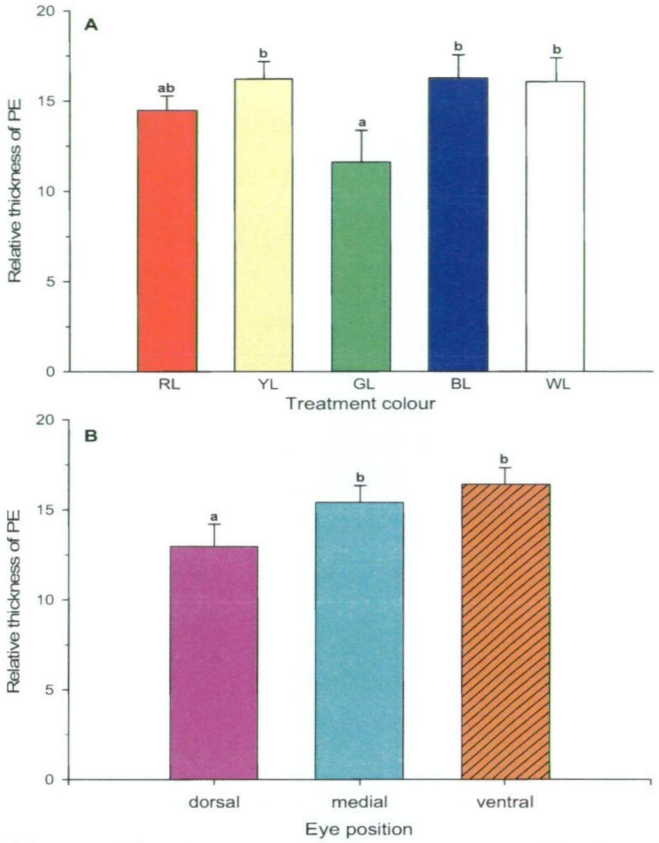

Figure 4.10: Relative thickness of the pigment epithelium for the light (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days, in relation to A: lighting colour and B: position in the eye.

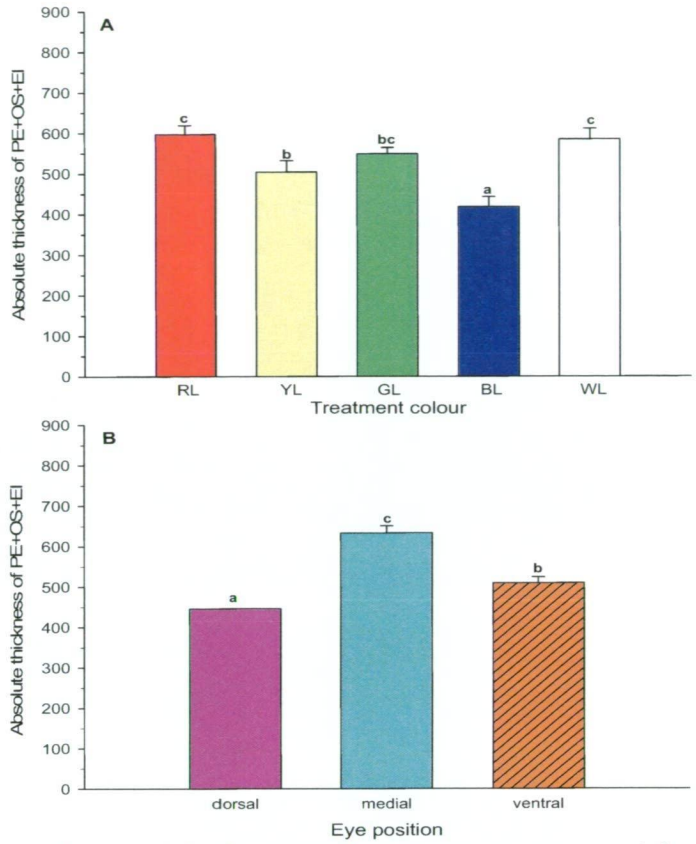

Figure 4.11: Absolute thickness of the pigment epithelium, outer segments and the ellipsoids for the light (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days, in relation to A: treatment colour and B: position in the eye.

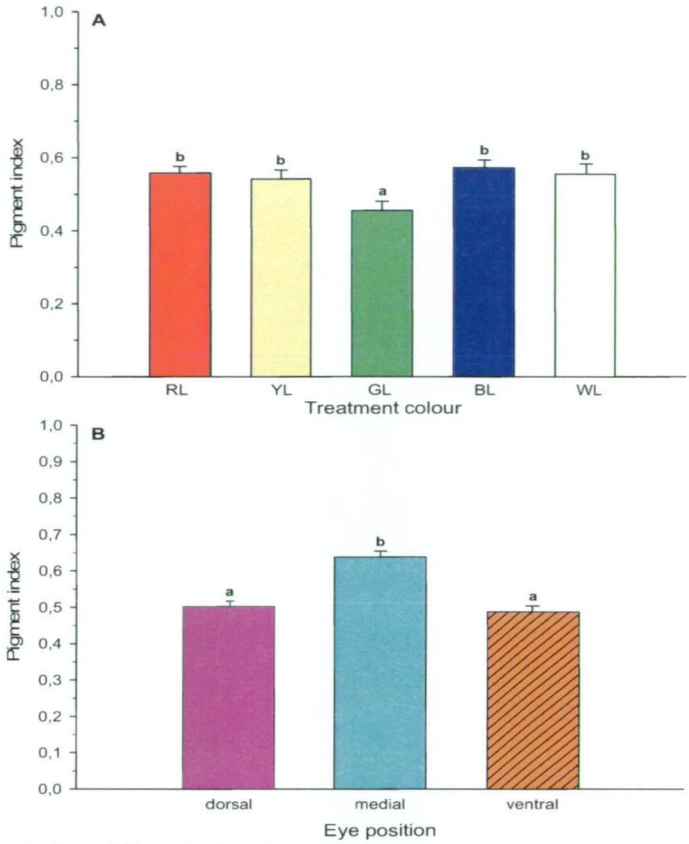

Figure 4.12: Pigment index of light (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days, in relation to A: lighting colour and B: position in the eye.

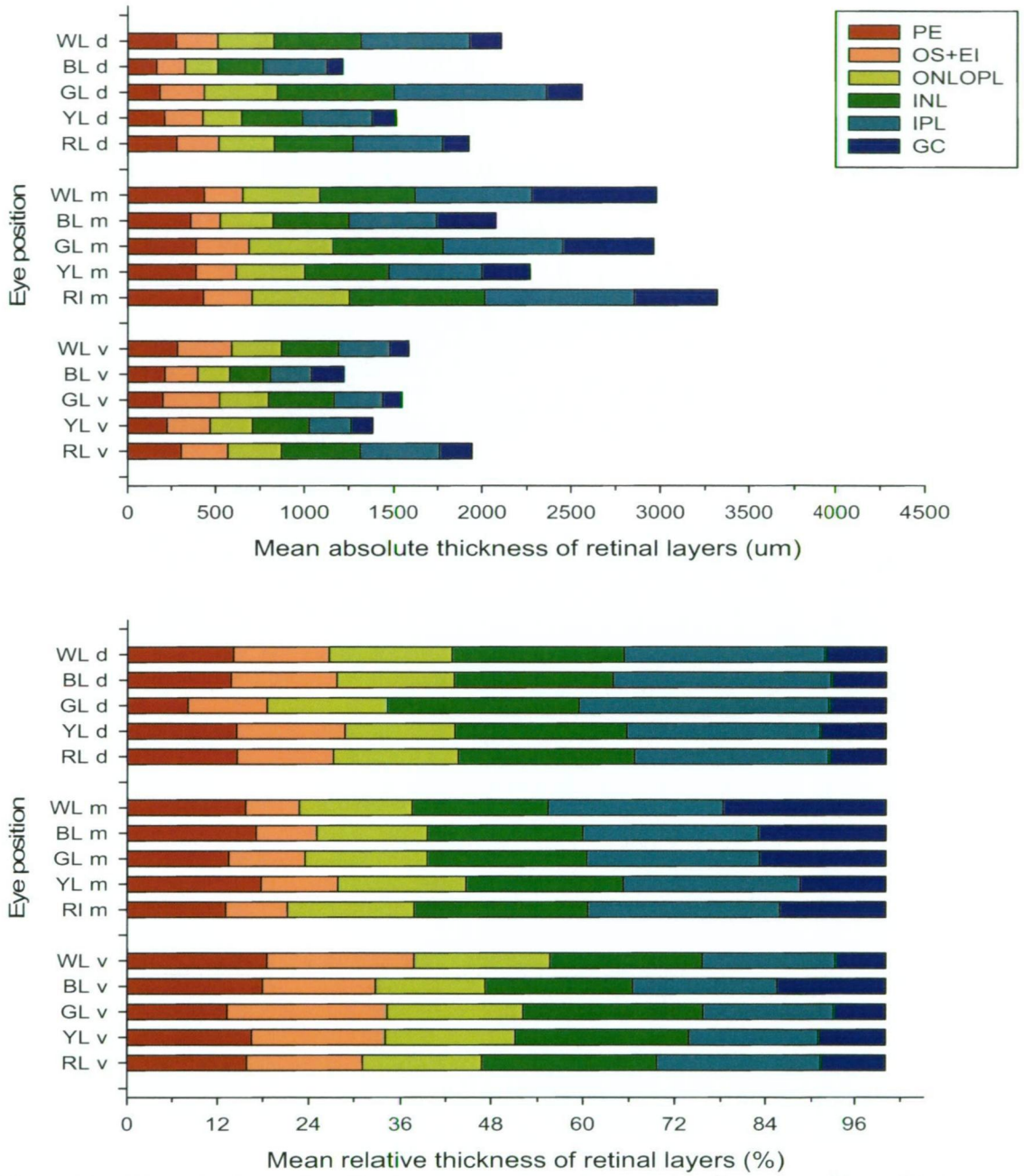

Figure 4.13: Mean absolute and relative layer thicknesses of light (red, yellow, green, blue, white) adapted *H. abdominalis* over a period of 56 days (d = dorsal; m = medial; v = ventral).

Table 4.2: Relative thickness (means ± SE) of retinal layers for all adaptation colours and eye positions of light adapted seahorse. F and P (df 14, 125); 2 - way ANOVAS (treatment colour/ eye position).

Light adaptation colour		Red	Yellow	Green	Blue	White	F	P	df
Relative thickness of retinal layers (%) (mean + SE)									
PE	**dorsal**	15 ± 1	14 ± 2	8 ± 3	14 ± 2	14 ± 3			
	medial	13 ± 2	18 ± 3	13 ± 3	17 ± 2	16 ± 4			
	ventral	16 ± 1	16 ± 2	13 ± 2	18 ± 3	19 ± 5	1.33	0.238	
OS + EI	**dorsal**	13 ± 1	14 ± 1	10 ± 1	14 ± 2	13 ± 3			
	medial	8 ± 0	10 ± 1	10 ± 0	8 ± 0	7 ± 1			
	ventral	15 ± 4	18 ± 1	21 ± 1	15 ± 3	19 ± 1	2.03	0.050	
ONL + OPL	**dorsal**	16 ± 1	14 ± 1	16 ± 1	15 ± 2	16 ± 3			
	medial	17 ± 1	17 ± 1	16 ± 1	14 ± 1	15 ± 1			
	ventral	16 ± 0	17 ± 1	18 ± 0	14 ± 0	18 ± 2	1.15	0.339	
INL	**dorsal**	23 ± 1	23 ± 1	25 ± 1	21 ± 0	23 ± 2			
	medial	23 ± 1	21 ± 0	21 ± 0	20 ± 1	18 ± 0			
	ventral	23 ± 1	23 ± 3	24 ± 2	19 ± 2	20 ± 1	1.06	0.399	
IPL	**dorsal**	26 ± 3	26 ± 2	33 ± 1	29 ± 4	27 ± 5			
	medial	25 ± 1	23 ± 1	23 ± 1	23 ± 3	23 ± 3			
	ventral	22 ± 3	17 ± 1	18 ± 3	19 ± 4	18 ± 1	1.62	0.127	
GC	**dorsal**	8 ± 1	9 ± 1	7 ± 1	7 ± 1	8 ± 1			
	medial	14 ± 4	12 ± 1	17 ± 4	17 ± 4	22 ± 8			
	ventral	9 ± 2	9 ± 2	7 ± 1	15 ± 8	7 ± 1	1.20	0.304	

PE = Pigment epithelium; OS = outer segments; EI = Ellipsoids; ONL = outer nuclei layer; OPL = outer plexiform layer; INL = inner nuclei layer; IPL = inner plexiform layer; GC = ganglion cells.

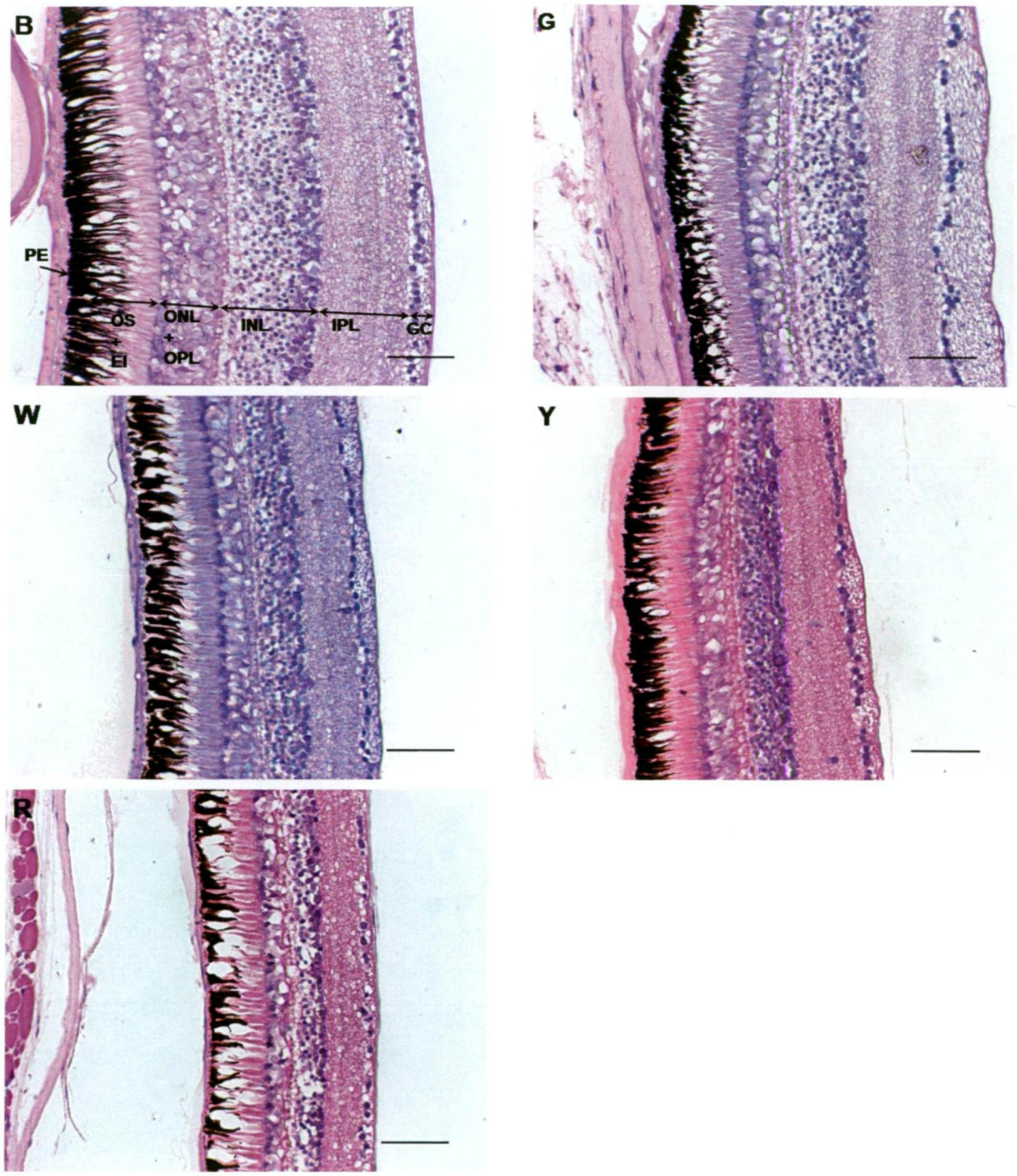

Figure 4.14: Transverse planes of medial sections of the eyes of background colour adapted *H. abdominalis* (R = red, Y = yellow, G = green, B = blue and W = white), over a period of 56 days, showing the differences in layer thickness in the retina due to the adaptation to different colours. PE = pigment epithelium, OS + EI = outer segments and ellipsoids, ONL + OPL = outer nuclei layer and outer plexiform layer, INL = inner nuclei layer, IPL = inner plexiform layer, GC = ganglion cell layer. Scale bar 50 μm.

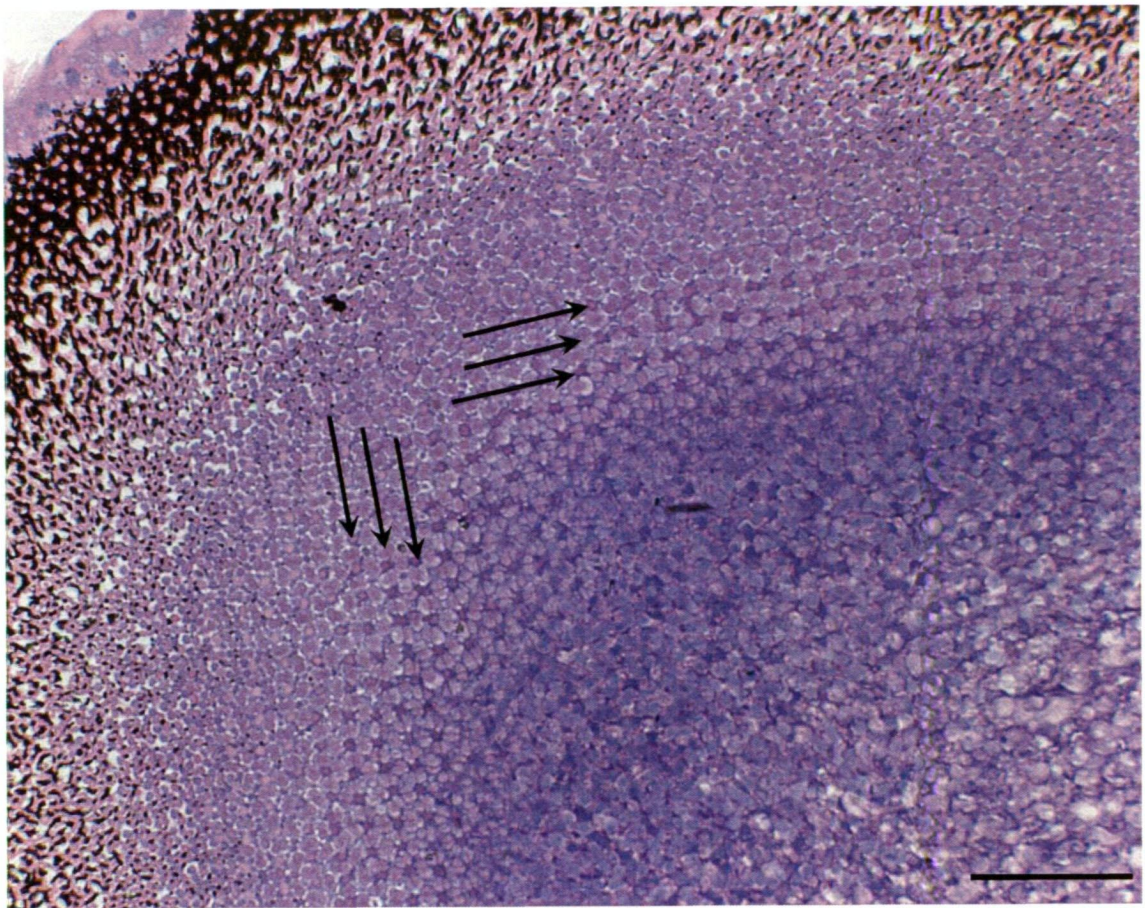

Figure 4.15: Retinal mosaic of *H. abdominalis* eye in sagittal section, showing rows of square units. Each arrow indicates one row. Scale bar 50 μm.

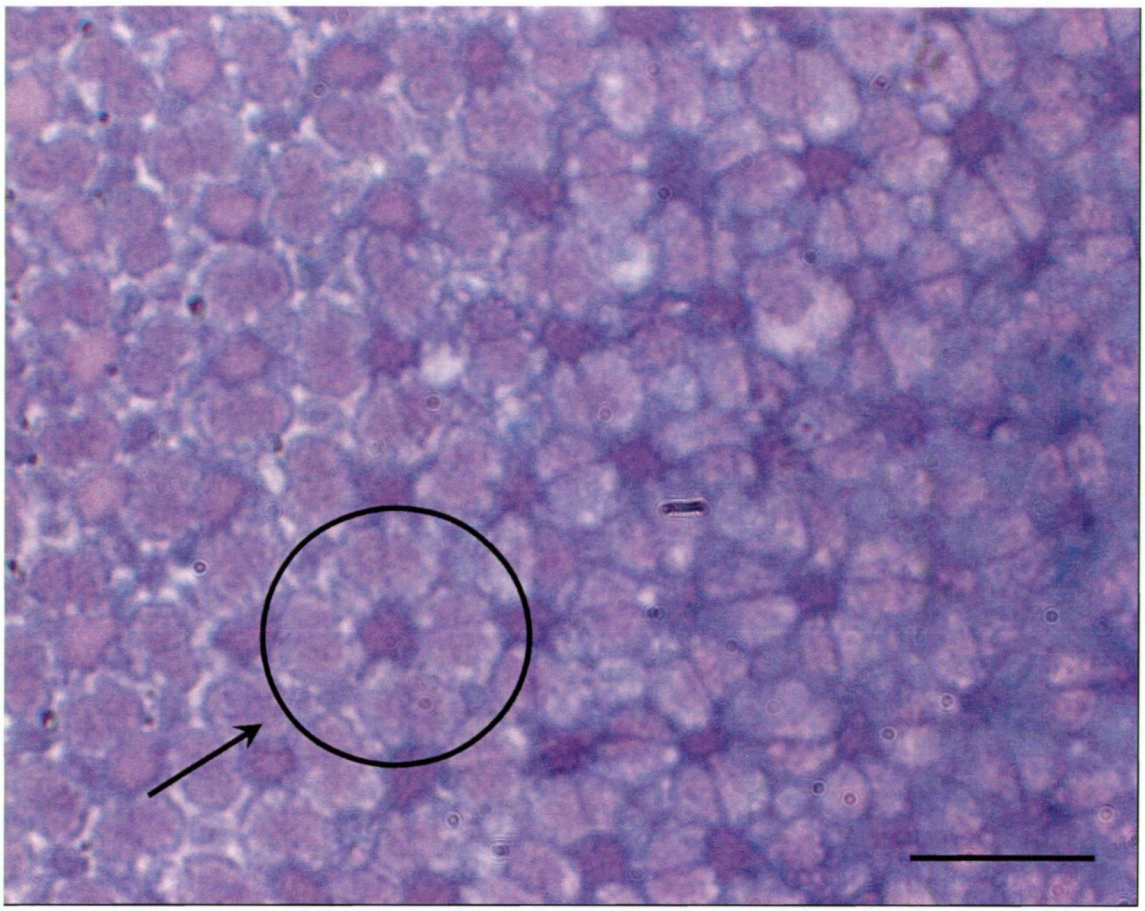

Figure 4.16: Square units of the mosaic of *H. abdominalis* eye in sagittal section, each unit containing 4 double cones and one single cone in the middle of the double cones. Scale bar 12.5 μm.

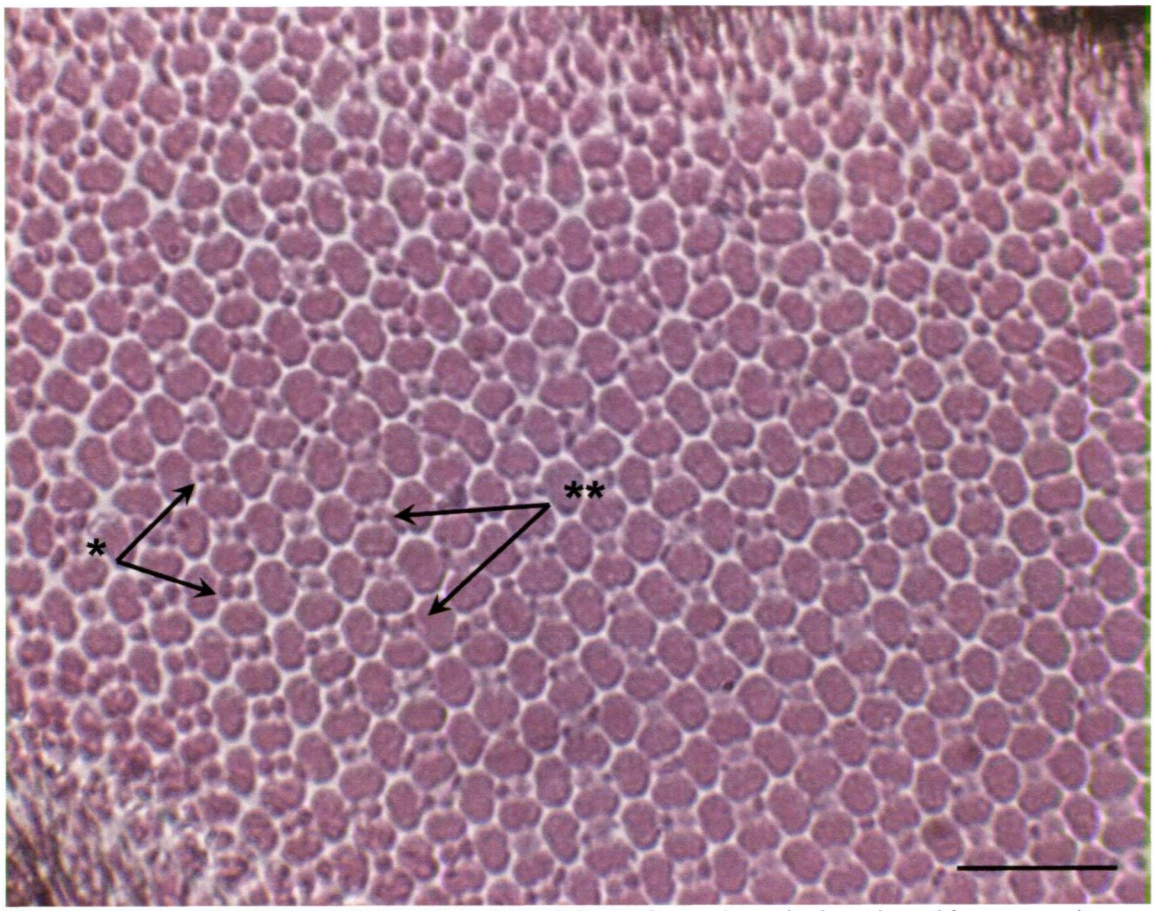

Figure 4.17: Irregular mosaic of blue adapted *H. abdominalis* eye in sagittal section with square units with 2 (*) or 3 (**) single cones in the middle of a unit. Scale bar 25 μm.

4.4. Discussion

The eye structure of *H. abdominalis* contained all normal retinal elements as described for syngnathidae (Engström 1963; Wagner 1971; Ali and Anctil 1976; Collin and Collin 1995; Mosk 2004) with mosaics formed mostly of square units of double and single cones (Engström 1963; Wagner 1971; Douglas and Djamgoz 1990). The medial section of the eye had the highest cone densities in all fish adapted to both different background and lighting colours. Fish adapted to red backgrounds had significantly lower cone density in the medial section than yellow, green, blue and white adapted fish, and yellow adapted fish had the significantly highest cone density. The cone density of the ventral section of eyes of red and blue light adapted fish was significantly lower than the cone density in green adapted fish.

Different light spectral environments also caused a change in the retinas of blue adapted fish, leading to two or three single cells in the middle of a mosaic unit where all other fish had one single cone in the middle of a unit (Engström 1963) (Fig. 4.17). The fish adapted to blue backgrounds and lights produced additional single cones, which are suggested to be blue sensitive (Douglas and Djamgoz 1990; Mosk 2004), and found in other species to improve vision in the blue environment (Boehlert 1978; Wagner 1990; Lara 2001) to be able for example to detect prey or to orientate in the new environment (Pankhurst and Eagar 1996). This finding provides additional support to the theory that stimulation of particular cone types with light corresponding to their spectral sensitivity, alters the differentiation of cone cells and orientation in mosaics (Munz and McFarland 1973).

The differences in cone density between the 5 colour treatments could have occurred due to differences in cone sensitivity. Mosk (2004) measured the spectral sensitivity of cones of *H. subelongatus* and found that orange–red sensitive double cones and blue sensitive single cones were far less abundant than green or green–yellow sensitive

double cones. The decrease in cone density in the present study of RB, RL and BL adapted fish and increase of density in YB and GL adapted fish in this study could be explained by differentiation of cones to spectra and therefore change in densities (Helvik et al. 2001). In the present study, adaptation to red or blue could have suppressed the development of cones which are sensitive to green and yellow light (Wagner 1990; Lara 2001) because of reduced stimuli at those wavelengths and therefore the cone densities declined. In addition numbers of green and green–yellow sensitive cones may naturally be higher in *H. abdominalis* as for *H. sublongatus*, leading to the significantly higher cone density in fish adapted to YB and GL, potentially enhanced by a decline of the few red and blue sensitive cones due to reduced stimuli. Wagner and Kröger (2005) also found that the retina reacts to changes of the spectral environment and the visual system responds with adaptive processes to the spectral composition of the surroundings. In an investigation of the visual pigments of juvenile marine fishes, Britt (2001) observed that green sensitive cones are dominant in species of inshore waters correlating with the light conditions in which they live and that benefits their visual behaviour, including foraging (Novales Flamarique and Hawryshyn 1993). Helvik offered similar results when he examined the photoreceptors of Atlantic halibut (*H. hoppoglossus*) where 90% of the photoreceptors in the retina are green sensitive and the other 10% express blue– and red–sensitive opsins. Additional research involving measurement of the light spectra and the spectral sensitivity of cones from the retinas of seahorses from each treatment colour is required to determine colour sensitivity in seahorses.

The medial section of the eye had the highest cone densities in both treatments, and probably comes as result of the eye orientation in the head of *H. abdominalis* (Mosk 2004), and the angle at which the light was reflected from the tank walls.

The measurement of retinal layer thickness showed that the retina of green–adapted fish was most influenced by adaptation colour (Fig. 4.15). The absolute thickness of the total retina was the thickest in green background adapted fish and green light adapted fish

also had a thick retina. The same pattern occurred in the absolute thickness of the PE+OS+EI, leading to green–adapted fish having a longer light pathway through the retinal layers than fish adapted to other colours. The relative thicknesses of the pigment epithelium within the retina in green–adapted fish were lower than in fish adapted to red, yellow, blue or white, resulting in a lower pigment index. Together, this indicates that fish adapted to the colour green maximised the capacity to capture light, suggesting that cone sensitivity was low in relation to the particular wavelengths they were exposed to in the experiment. A thick retina and a small degree of ellipsoid shading suggests that more information had to be processed to cope in the new environment for example to see prey, and that the actual energy from the quantity of received light was probably lower in green than in the other 4 test colours. This could have happened due to a longer light pathway or a poor reflecting surface in the background adaptation system and low levels of energy (less than 0.8 relative intensity; see Appendix C) in a narrow range of wavelengths (520-550 nm = green, (Sylvania 2004) in the light adaptation system. An investigation of the retinal structures of bird species showed that birds like the American robin (*Turdus migratorius*) which start singing early in the morning have thicker retinal layers. Especially a thicker ganglion cell layer suggesting that a greater number of ganglion cells maximize vision under low light conditions (McNeil et al. 2005).

Further studies are required to investigate whether the stimuli of energy around this wavelength will lead to a development of cones sensitive to a specific spectral range or whether fish increase the density (or number) of cone numbers of a specific sensitivity to optimise vision where overall photoreceptor sensitivity is low.

Chapter 5

General Discussion

5.1. General Discussion

This study examined whether coloured tanks or coloured artificial light influence skin colour of fish, and investigated potential influences of culture conditions on the fish's vision (colour preferences) and retinal structure. Fish were held in different coloured tanks or under different coloured lights (red, yellow, green, blue, white) for a period of 56 days and their skin colour was measured every fortnight. Colour preferences (background, lighting) of non–adapted fish of different life stages as well as adapted fish were tested. After 56 days, fish held under these conditions were sampled for histological analysis of their retinal eye structures and cone mosaics.

From these experiments, it appears that exposure to coloured backgrounds had a greater influence on seahorse skin colour than exposure to coloured light. Seahorses held in different coloured tanks were able to change their skin colour to "adapt" to their new environments and their background colour preferences changed favourably towards the colour under which they were held. The market–preferred golden–yellow colouration of *H. abdominalis* was achieved by holding the fish in yellow tanks. These holding conditions also influenced their background colour preference for yellow to be greater than in non–adapted fish. Light adaptation and lighting colour however, did not influence skin colour or colour preferences to a great extent, suggesting that body colouration changes are best achieved with background adaptation. The different response in skin colouration to the two colour treatments (background, lights) on seahorses may be due to the different ways colour was received by the seahorse eye. In the background treatments white light was reflected from the coloured tank walls and base underwater before the colour was received by the eye. In contrast, overhead coloured light instead travelled into the tanks and was received directly by the eyes. In aquatic environments the direction from which light comes is important as in water light is absorbed and scattered on the path to the eye (Vorobyev et al. 2001). The position of the eye relative to the fish's body determines from which direction light is received. Seahorses which have eyes that move independently of each other and are situated

laterally on their head (Woods 2003b) are mostly influenced by their backgrounds (habitat). In this experiment backgrounds (tank walls) reflect light from the surroundings, which enters the seahorse eyes directly, falling onto the central region of the retina where the highest density of cones cells are located (Mosk 2004) and colour can be detected. That gives seahorses the chance of trying to match the background colouration to avoid predation (Kuiter 2000). Overhead light may likely reach only the ventral region of the retina containing fewer cone cells (Mosk 2004) and therefore the coloured light did not have a significant influence on either seahorse skin colour or on their colour preferences.

Seahorses from the initial population that had been held under standard conditions had a preference for a white background and for green, blue and white lighting colours. Red and yellow backgrounds and lights were avoided by the non–adapted seahorses in the background preference test as well as in the light preference test. Previous studies support these findings where it has been shown that most marine and freshwater species prefer green and blue backgrounds and lights (Kawamoto and Takeda 1950; 1951; Kawamura et al. 1996; Maaß 2004; Maaß et al. unpublished data) because these are similar to the colouration of their natural environment (Lythgoe 1968; 1984; 1985; Bowmaker 1990). Pot bellied seahorses have been shown to avoid dark background colours (Woods 2000a) which supports the preference for white in this experiment.

In seahorses not much is known about the visual capacity, their eye development and retinal structure of the 35 seahorse species living in very diverse environments. (Engström 1963; Wagner 1971; Ali and Anctil 1976; Mosk 2004). Studies on fish retinas have recently been concentrated on juvenile development (Shand et al. 2002; Cobcroft and Pankhurst 2003; Carvalho et al. 2004; Evans and Browman 2004; Jones and Kaiser 2005; Cobcroft and Pankhurst 2006), or spectral sensitivity (Reckel et al. 2002; Collin et al. 2003; Fritsches et al. 2003; Losey et al. 2003; Mosk 2004; Matsumoto and Kawamura 2005; Novales Flamarique 2005; Pointer et al. 2005) but to my knowledge no studies have been investigated links between retinal changes and

environmental influences. In the present study, histological examination of the retinas supported that the direction of how light is reflected from the tanks and received by the fish's eye plays an imported role on the cone density of the retinal regions. Colour had the greatest influence on cone density in the medial region of the eye of background adapted fish and on the cone density of the ventral region in light adapted fish.
The retinomotor response expressed by the pigment index showed that the dorsal and medial parts of the eye in background adapted fish were significantly more shaded than the ventral region which means that the dorsal and medial section of the eye were more stimulated by light. In the light adapted fish however, no differences in the shading of the dorsal and ventral region of the eye were found which leads to the conclusion that all regions of the eye were equally stimulated by light.

Fish that were adapted to a green background had a thicker retina than other fish and therefore a longer light pathway. The relative width of the retinal pigment epithelium however was thinner in green background adapted fish and in the medial region of green light adapted fish, than in fish from the other treatment colours. The width of the pigment epithelium may have been influenced by the light spectrum the fish received from their environment. However there is very little information available (Helvik et al. 2001; Cheng et al. 2006) relating retinal structure to specific coloured environments. Furthermore it is unclear whether the light perceived relates to the spectra offered by light or reflected from the tank walls.

The results of this study suggest that there is potential for commercial farms to produce more colourful seahorses for the aquarium trade by keeping them in coloured tanks. Which of the colour morphs found in this study are the most ideal for the aquarium trade has to be evaluated by the industry and there is potential to use different tank colours to produce other colour morphs. The ability of skin colour to change appears closely related to the recognition of visual cues and capability of the fish to process the cues. As shown in this study, adaptation to a yellow background changed the fishes skin colour, but also influenced their natural background colour preference. Initial (non–

adapted) fish avoided the yellow background colour in the background colour preference–test whereas fish previously adapted to yellow had a positive preference for the yellow background colour when reexposed to a choice of colour. Furthermore, adaptation has resulted in an altered retinal structure according to the holding colour. Therefore, caution should be taken if plans for restocking programs are undertaken in the future because tank and lighting conditions on farms could influence the fish's development to an extent where they are either not appropriately camouflaged against predators or the visual system may be altered after acclimatised to artificial light to a degree where fish may have problems orientating and capturing prey in a natural light environment (Olla et al. 1994; Olla et al. 1998; Brown and Laland 2001; Brown et al. 2003; Braithwaite and Salvanes 2005; Santos et al. 2006). Therefore, there should be specific culture conditions for fish for the ornamental trade and those for restocking programmes. Fish for planned restocking programmes should be bred and held in the most natural environment as possible to avoid problems, like starvation, disorientation and predation in the natural habitat after restocking.

5.2. Future Directions

Future research on skin colour changes in seahorses should include studies on long term adaptation to different tank colours and the reversibility of the skin colour change. Ideally fish should be kept in coloured tanks from birth until their sexual maturity is reached and then be transferred to normal aquariums to see if the skin colour stays the same or fades over time. This would give commercial farmers an indication of which colours of fish are going be the most successful for the aquarium trade or if consumers have to apply coloured objects or backgrounds in their aquariums to maintain the skin colour of their seahorses.

Secondly, an understanding of the changes in pigment quantity and variety after adaptation could provide further information on the mechanisms and ability of

seahorses, *H. abdominalis*, to undergo morphological colour change. Therefore, skin pigments and their quantities should be examined, for example with a photo spectrometer analysis, in non–adapted fish and in fish which have changed their skin colour during background adaptation.

Another suggestion is to run a selective breeding program with naturally colourful brood stock to determine if genetics plays an important role in actual skin colour or the ability of the skin to change colour. This could be done by selecting male and female fish with the desired skin colour, breeding them and analysing the variety of different colour morphs in the juveniles. Fish with desirable colours can then be used in a selective breeding program.

Of further interest may also be the role of retinal changes in fish which have been adapted to artificial environments. This is of high interest for all species for which restocking programs exist to ensure that the farming conditions do not have an influence on the performance of the fish after restocking. Therefore, retinal structures like cone densities and retinal layer thicknesses of wild and farmed fish throughout their development should be examined. These samples should then be compared to each other to determine if the farming conditions changed or influenced the eye structures.

Finally, determining the colour preferences of fish species could be beneficial for the fish welfare on commercial land based fish farms. By knowing the species natural colour preferences, farms could optimise culture conditions for the fish, accounting for lighting colour, light intensities and tank colours, which may reduce stress, improve health and promote better growth.

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Appendices

Appendix A: Colour charts and Body colouration of fish after adaptation

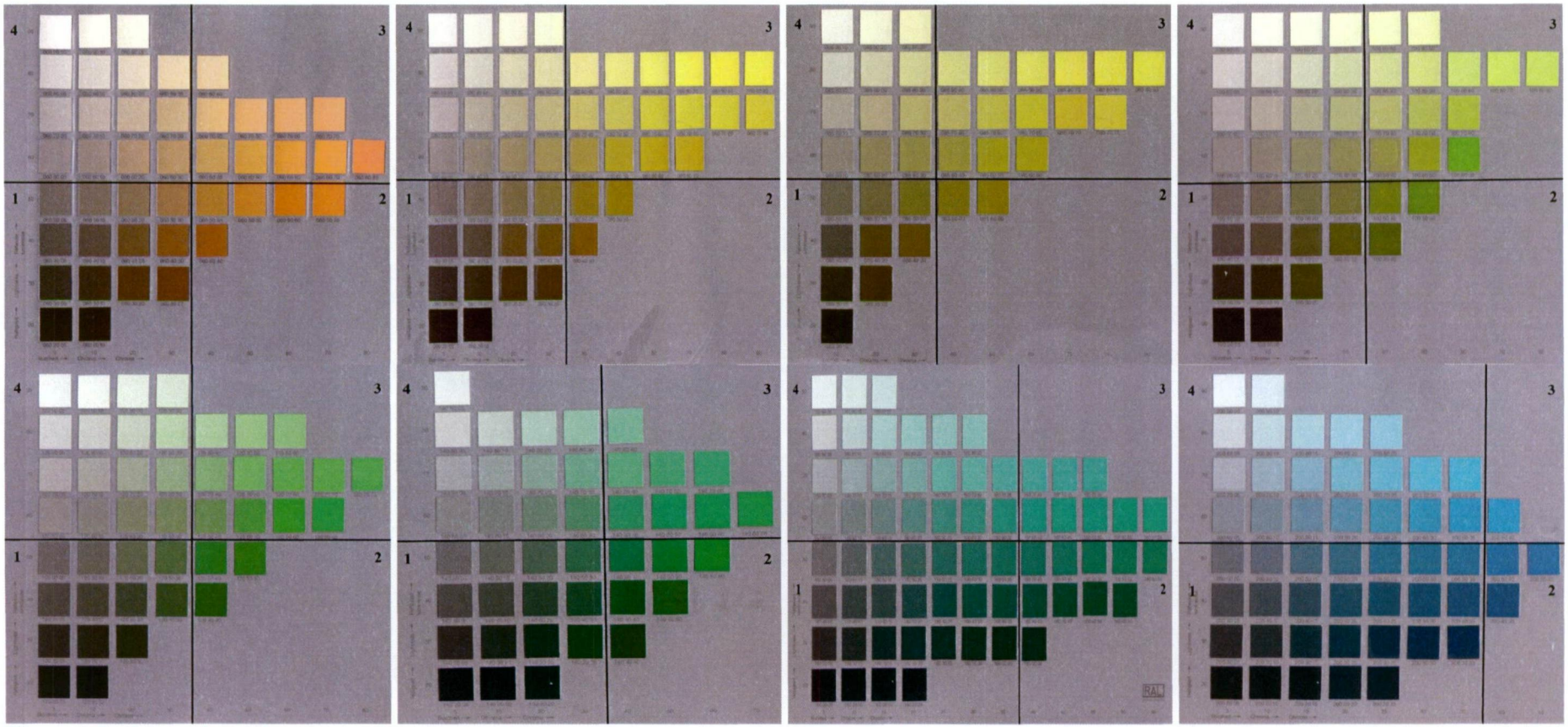


Figure A 1: Colour charts, that were used to measure skin colouration (hues: 060, 080, 085, 100, 120, 140, 160, 200 from left to right and top to bottom) with sectors 1, 2, 3 and 4 (anticlockwise beginning at the left lower side).

Table A 1: Sections of the colour chart for the different hues of ventral colouration with numbers of fish per section and tank colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	0	0	7
	070	0	1	1	0
	080	2	0	2	48
	085	3	1	0	0
	160	0	0	0	0
Yellow	060	0	2	0	6
	070	1	5	0	1
	080	2	8	5	33
	085	2	4	0	0
	160	0	0	0	0
Green	060	0	1	0	5
	070	0	0	0	0
	080	4	3	1	44
	085	2	0	0	5
	160	0	0	0	0
Blue	060	0	2	0	1
	070	0	0	0	0
	080	15	11	3	29
	085	3	3	0	3
	160	0	0	0	0
White	060	1	0	0	0
	070	2	0	0	0
	080	20	6	5	27
	085	4	4	0	3
	160	1	0	0	0

Table A 2: Sections of the colour chart for the different hues of dorsal colouration with numbers of fish per section and tank colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	0	0	2
	070	0	1	0	0
	080	0	0	0	63
	085	0	0	0	0
Yellow	060	0	0	0	4
	070	0	0	0	0
	080	1	0	1	61
	085	1	1	0	1
Green	060	0	0	0	1
	070	0	0	0	0
	080	0	0	0	62
	085	0	0	0	2
Blue	060	0	1	0	2
	070	0	0	0	0
	080	5	0	2	60
	085	0	0	0	2
White	060	0	0	0	0
	070	0	0	0	0
	080	2	0	0	67
	085	0	0	0	4

Table A 3: Sections of the colour chart for the different hues of spot colouration with numbers of fish per section and tank colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	1	0	0
	070	0	0	0	0
	080	5	0	1	27
	085	2	1	0	0
	100	10	1	0	0
	120	1	1	0	0
	140	10	0	0	0
	160	2	0	0	1
	200	3	0	0	0
Yellow	060	0	0	0	3
	070	0	2	1	0
	080	15	2	0	34
	085	1	0	0	4
	100	0	1	0	0
	120	0	0	0	0
	140	2	0	0	1
	160	2	0	0	0
	200	2	0	0	0
Green	060	0	0	0	2
	070	0	0	0	0
	080	12	0	0	20
	085	1	0	0	2
	100	3	4	0	1
	120	3	2	0	2
	140	2	1	0	0
	160	5	0	0	1
	200	1	0	0	0
Blue	060	0	1	0	0
	070	0	0	0	0
	080	14	3	0	34
	085	0	0	0	3
	100	0	0	0	0
	120	1	0	0	2
	140	5	0	0	0
	160	4	0	0	0
	200	3	0	0	0
White	060	0	0	0	0
	070	0	0	0	0
	080	12	1	0	42
	085	0	1	0	1
	100	1	0	0	0
	120	1	2	0	1
	140	3	0	0	1
	160	5	0	0	0
	200	1	0	0	0

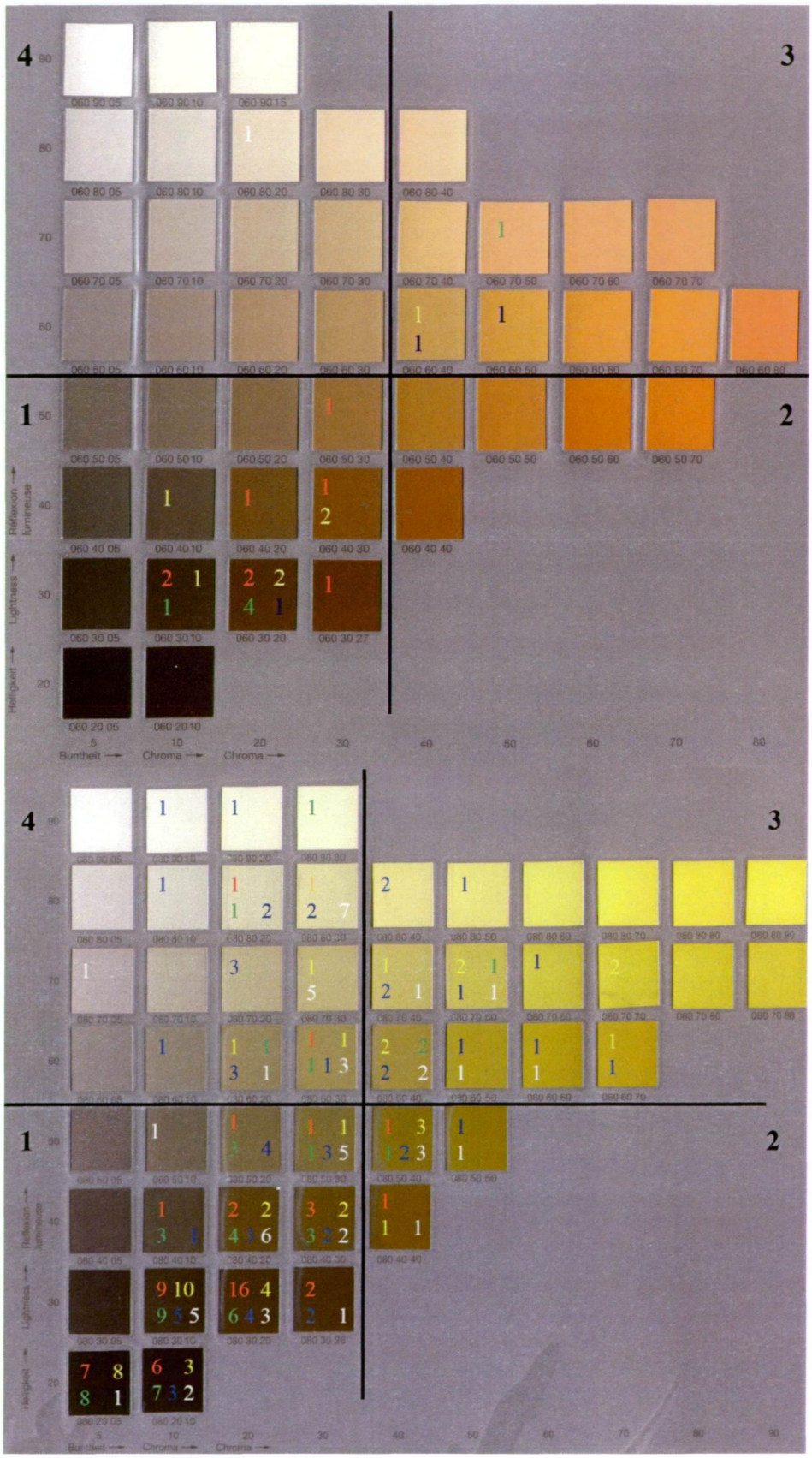


Figure A 2: Colour charts 060 and 080 with the ventral colouration of all individuals of the background adaptation trial day 56 displayed as an example of colour distribution.

Table A 4: Sections of the colour chart for the different hues of ventral colouration with numbers of fish per section and light colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	0	0	0
	070	0	0	0	0
	080	6	0	0	42
	085	2	1	0	2
Yellow	060	0	0	0	3
	070	0	0	0	0
	080	6	3	0	29
	085	6	1	0	2
Green	060	0	0	0	0
	070	0	0	0	0
	080	7	2	0	32
	085	1	0	0	3
Blue	060	0	2	0	1
	070	0	0	0	0
	080	9	2	2	39
	085	2	0	0	1
White	060	0	0	0	1
	070	0	0	0	0
	080	8	2	2	27
	085	2	2	0	1

Table A 5: Sections of the colour chart for the different hues of dorsal colouration with numbers of fish per section and light colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	0	0	0
	070	0	0	0	0
	080	0	0	0	53
	085	0	0	0	0
Yellow	060	0	0	0	1
	070	0	0	0	2
	080	0	0	0	46
	085	0	0	0	1
Green	060	0	0	0	1
	070	0	0	0	0
	080	0	0	0	44
	085	0	0	0	0
Blue	060	0	0	0	2
	070	0	0	0	1
	080	0	0	0	54
	085	0	0	0	1
White	060	0	0	0	1
	070	0	0	0	0
	080	0	0	0	44
	085	0	0	0	0

Table A 6: Sections of the colour chart for the different hues of spot colouration with numbers of fish per section and light colours they were adapted to.

		Number of fish in section			
tank colour	Hue	4	3	2	1
Red	060	0	0	0	0
	070	0	0	0	0
	080	5	0	0	33
	085	0	0	0	0
	100	2	0	0	0
	120	3	0	0	0
	140	0	0	0	0
	160	7	0	0	0
	200	3	0	0	0
	220	0	0	0	0
Yellow	060	0	0	0	2
	070	0	0	0	1
	080	2	0	0	34
	085	1	1	0	2
	100	1	1	0	0
	120	1	1	0	0
	140	0	0	0	0
	160	1	0	0	0
	200	2	0	0	0
	220	0	0	0	0
Green	060	0	0	0	0
	070	0	0	0	0
	080	10	0	0	20
	085	0	0	0	0
	100	0	0	0	0
	120	0	0	0	3
	140	0	0	0	0
	160	3	0	0	1
	200	5	0	0	0
	220	1	0	0	0
Blue	060	0	0	0	1
	070	0	0	0	1
	080	8	0	0	33
	085	0	0	0	1
	100	2	1	0	0
	120	1	0	0	0
	140	0	0	0	0
	160	5	0	0	1
	200	4	0	0	0
	220	0	0	0	0
White	060	0	0	0	0
	070	0	0	0	0
	080	4	0	0	20
	085	0	1	0	0
	100	1	1	0	3
	120	0	1	0	3
	140	0	1	0	0
	160	5	0	0	1
	200	4	0	0	0
	220	0	0	0	0

Appendix B: Photographs of different colour morphs

Figure B 1: Colour example for a red adapted fish having irridescent green stripes.

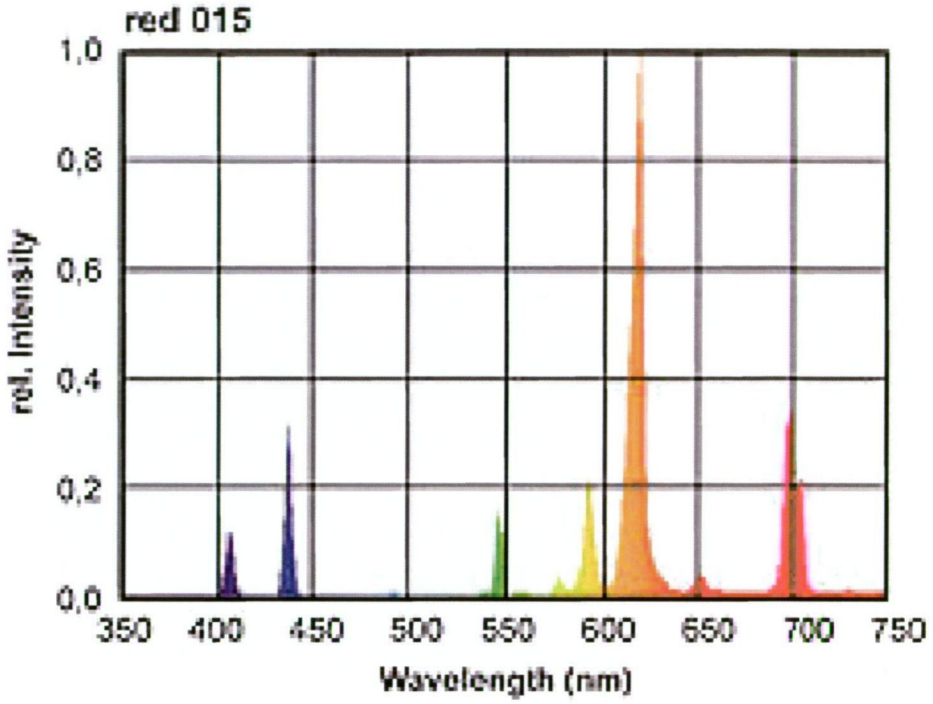
Figure B 2: Colour example for a yellow adapted fish with a light yellow ventral colouration and brown dorsal colouration.

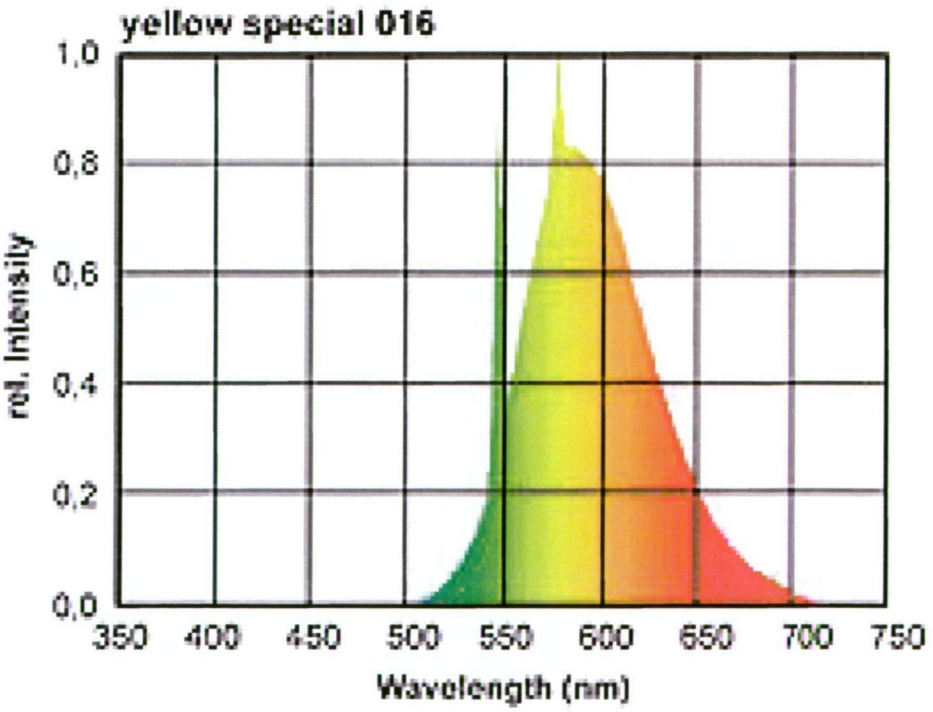
Figure B 3: Colour examples for green adapted fish with light green spots (top) and turquoise spots (bottom).

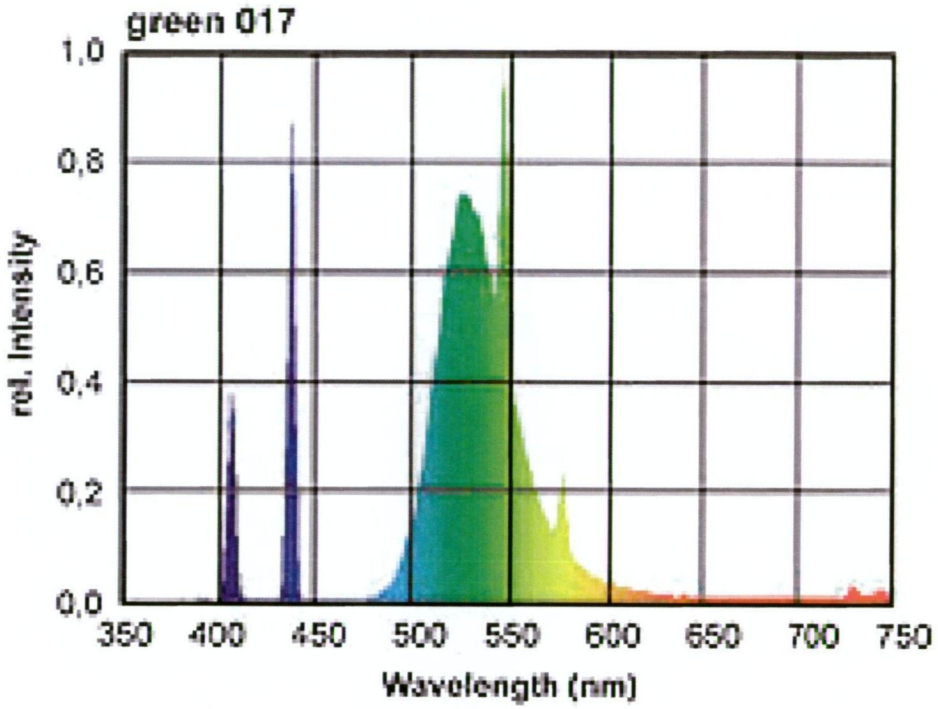
Figure B 4: Colour example for a blue adapted fish with a lighter ventral colouration and a brown-greyish dorsal colouration.

Figure B 5: Colour example for a white adapted fish with a light yellowish-white ventral colouration and a light greyish-brown dorsal body part with brown spots.

Appendix C: Fluorescent lights



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0,6
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0,2
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rel. Intensity
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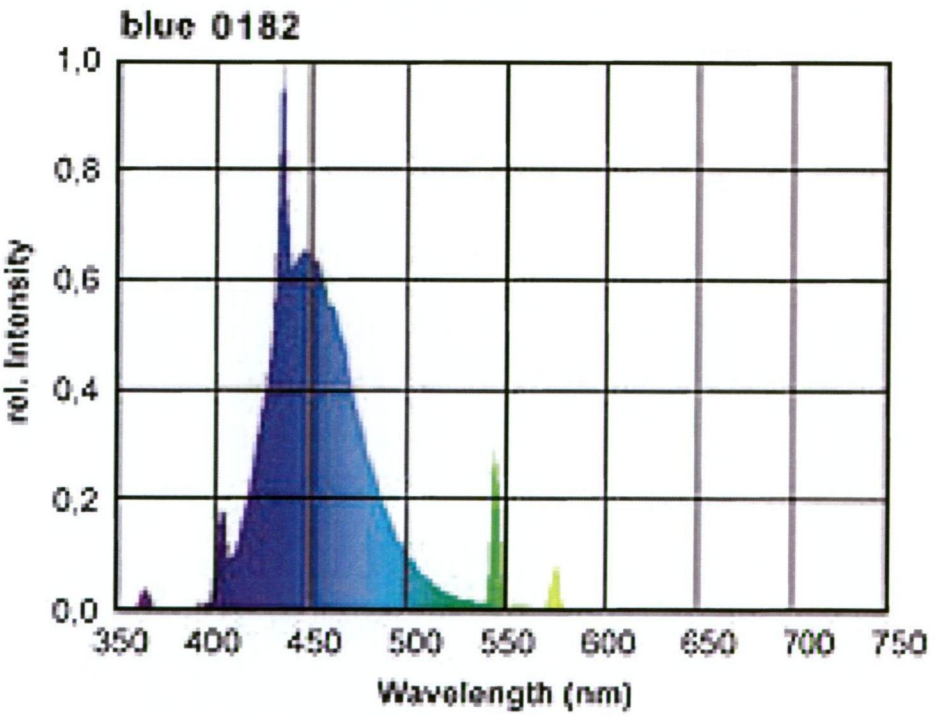
400
450
500
550
600
650
700
750
Wavelength (nm)


blue 0182
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0,8
0,6
0,4
0,2
0,0
rel. Intensity
350
400
450
500
550
600
650
700
750
Wavelength (nm)

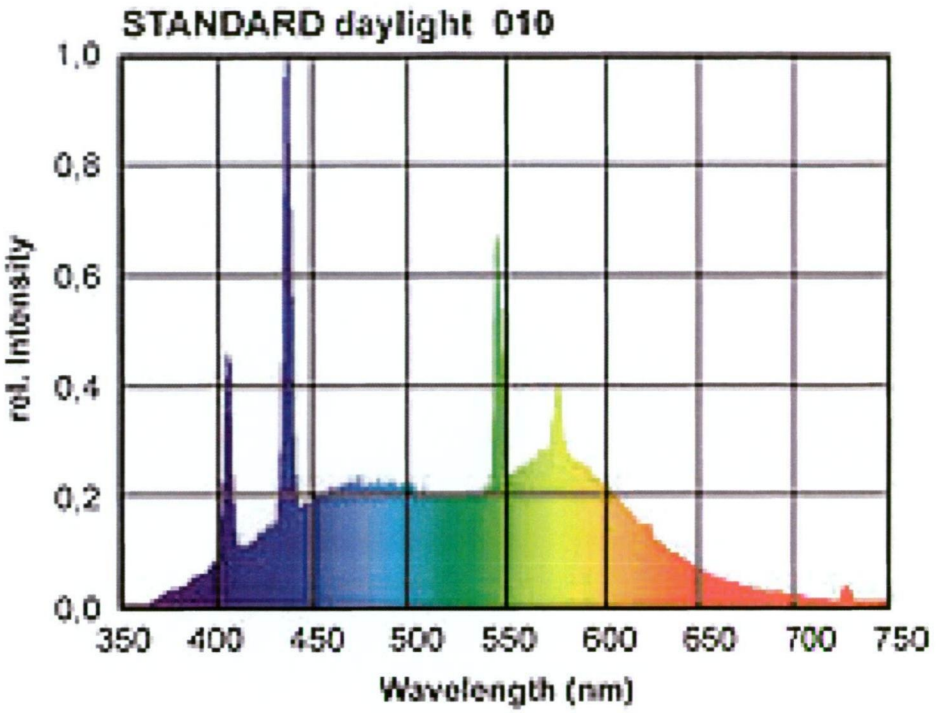

Figure C 1: Spectra of the coloured lights (red, yellow, green, blue and white, from top to bottom) used in the experimental section Chapter 2 (Sylvania 2004).

Appendix D: The eye of H. abdominalis

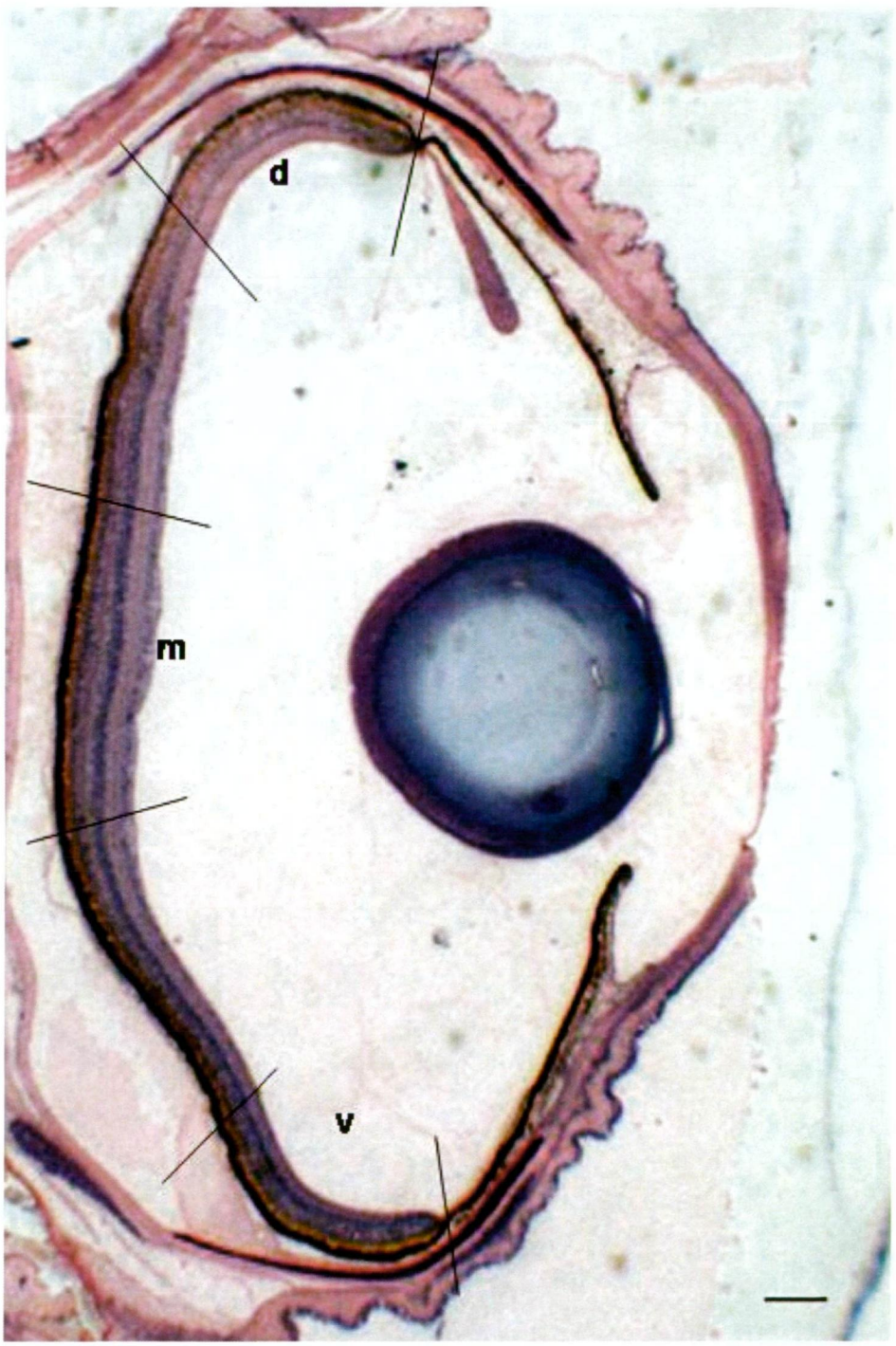

Figure D 1: Transverse section of the whole eye of the pot bellied seahorse H. abdominalis under 32 x magnification with dorsal, medial and ventral regions where measurements were taken. Scale bar = 200 μm.

Appendix E: Absolute thickness of retinal layers

Table E 1: Eye diameter, weight of fish and absolute thickness of retinal layers for all adaptation colours and eye positions of background adapted fish. In means ± SE, significance levels and F, 2 – way ANOVAS (treatment colour/ position).

Background adaptation colours		Red	Yellow	Green	Blue	White	F	P
Eye diameter (mm) (mean ± SE)		29.03 ± 2.22	31.69 ± 0.75	30.08 ± 1.32	32.39 ± 2.09	30.94 ± 0.35		
Weight of fish (g) (mean ± SE)		1.31 ± 0.20	1.70 ± 0.35	1.42 ± 0.26	1.75 ± 0.36	1.61 ± 0.39		
Absolute thickness of retinal layers (µm) (mean ± SE)								
PE	dorsal	350 ± 32	303 ± 28	394 ± 32	195 ± 19	337 ± 43		
	medial	484 ± 31	435 ± 18	381 ± 24	396 ± 31	344 ± 14		
	ventral	235 ± 21	210 ± 17	347 ± 15	261 ± 34	299 ± 32	5.05	0.000
PE + OS + EI	dorsal	640 ± 23	514 ± 27	748 ± 60	382 ± 29	586 ± 34		
	medial	761 ± 32	730 ± 30	762 ± 83	666 ± 62	585 ± 20		
	ventral	548 ± 29	452 ± 41	756 ± 37	505 ± 52	590 ± 47	3.48	0.001
ONL + OPL	dorsal	354 ± 32	305 ± 30	526 ± 21	238 ± 28	350 ± 37		
	medial	587 ± 58	610 ± 40	630 ± 47	524 ± 68	390 ± 25		
	ventral	350 ± 34	236 ± 24	463 ± 26	293 ± 40	303 ± 38	2.70	0.008
INL	dorsal	400 ± 37	330 ± 42	484 ± 27	339 ± 32	387 ± 50		
	medial	730 ± 74	559 ± 56	810 ± 56	669 ± 73	458 ± 55		
	ventral	403 ± 45	331 ± 39	423 ± 25	328 ± 42	257 ± 22	1.38	0.212
IPL	dorsal	377 ± 40	408 ± 45	621 ± 49	294 ± 38	486 ± 54		
	medial	728 ± 66	673 ± 37	893 ± 97	648 ± 65	462 ± 26		
	ventral	323 ± 37	230 ± 22	459 ± 78	321 ± 54	192 ± 17	2.63	0.010

GC	**dorsal**	128 ± 19	106 ± 10	153 ± 15	94 ± 6	152 ± 27		
	medial	309 ± 23	274 ± 16	585 ± 72	310 ± 28	452 ± 160		
	ventral	107 ± 13	79 ± 8	139 ± 19	116 ± 26	75 ± 5	2.48	0.015
WR	**dorsal**	1896 ± 134	1593 ± 127	2544 ± 114	1320 ± 115	1970 ± 195		
	medial	3105 ± 246	2927 ± 146	3646 ± 202	2859 ± 261	2358 ± 229		
	ventral	1716 ± 128	1276 ± 108	2237 ± 118	1540 ± 200	1411 ± 120	1.77	0.088
Cone counts								
Ellipsoids	**dorsal**	13.00 ± 0.75	12.58 ± 0.47	13.11 ± 0.70	12.08 ± 0.26	12.89 ± 0.81		
	medial	13.50 ± 0.56	15.50 ± 0.34	14.00 ± 0.65	14.42 ± 0.40	13.78 ± 0.55		
	ventral	12.00 ± 0.44	11.75 ± 0.30	11.67 ± 0.33	12.75 ± 0.39	12.22 ± 0.43	1.85	0.073
Nucleiis	**dorsal**	12.33 ± 0.73	12.25 ± 0.41	12.78 ± 0.64	12.00 ± 0.30	12.67 ± 0.88		
	medial	12.75 ± 0.45	14.75 ± 0.48	14.00 ± 0.55	14.92 ± 0.42	13.22 ± 0.66		
	ventral	12.25 ± 0.39	11.58 ± 0.36	11.22 ± 0.32	12.50 ± 0.40	12.22 ± 0.49	2.14	0.036

PE = Pigment epithelium; OS = outer segments; EI = Ellipsoids; ONL = outer nuclei layer; OPL = outer plexiform layer; INL = inner nuclei layer; IPL = inner plexiform layer; GC = ganglion cells; WR = thickness of whole retina.

Table E 2: Eye diameter, weight of fish and absolute thickness of retinal layers for all adaptation colours and eye positions of light adapted fish. In means ± SE, significance levels and F, 2 – way ANOVAS (treatment colour/ position).

Light adaptation colours		Red	Yellow	Green	Blue	White	F	P
Eye diameter (mm) (mean ± SE)		31.07 ± 0.44	32.41 ± 1.39	21.24 ± 1.83	33.27 ± 0.94	30.75 ± 1.85		
Weight of fish (g) (mean ± SE)		1.26 ± 0.12	1.08 ± 0.08	1.35 ± 0.30	1.60 ± 0.38	1.42 ± 0.32		
Retinal layers (µm) (mean ± SE)								
PE	**dorsal**	279 ± 16	210 ± 14	183 ± 15	164 ± 7	276 ± 28		
	medial	432 ± 31	391 ± 24	390 ± 31	360 ± 33	435 ± 36		
	ventral	306 ± 32	225 ± 15	201 ± 14	213 ± 17	283 ± 33	0.32	0.957
PE + OS + EI	**dorsal**	519 ± 14	429 ± 28	437 ± 4	329 ± 10	513 ± 14		
	medial	704 ± 30	614 ± 32	687 ± 26	525 ± 40	652 ± 38		
	ventral	569 ± 21	470 ± 24	523 ± 15	400 ± 25	588 ± 28	0.74	0.660
ONL + OPL	**dorsal**	313 ± 16	217 ± 19	141 ± 84	184 ± 5	315 ± 20		
	medial	550 ± 34	389 ± 46	470 ± 7	300 ± 15	433 ± 30		
	ventral	305 ± 36	237 ± 13	277 ± 14	180 ± 13	285 ± 33	1.85	0.076
INL	**dorsal**	441 ± 12	343 ± 34	652 ± 89	252 ± 10	491 ± 86		
	medial	763 ± 48	469 ± 43	622 ± 23	425 ± 24	533 ± 45		
	ventral	442 ± 47	321 ± 32	366 ± 27	229 ± 15	317 ± 24	3.21	0.003
IPL	**dorsal**	503 ± 50	392 ±50.	859 ±129	359 ± 42	608 ± 145		
	medial	837 ± 41	528 ± 52	673 ± 37	491 ± 57	658 ± 48		
	ventral	444 ± 79	235 ± 10	274 ± 37	230 ± 24	286 ± 31	2.85	0.007
GC	**dorsal**	146 ± 12	128 ± 6	203 ± 43	89 ± 12	180 ± 35		
	medial	462 ± 46	267 ± 35	510 ± 83	336 ± 50	701 ± 163		
	ventral	181 ± 37	124 ± 13	104 ± 10	184 ± 113	105 ± 14	2.68	0.010

WR	**dorsal**	1884 ± 92	1501 ±124	2509.24 ± 305	1221 ± 49	2116 ± 284		
	medial	3374 ± 148	2233 ± 189	2936.72 ± 127	2090 ± 91	2963 ± 236		
	ventral	1931 ± 217	1379 ± 61	1519.69 ± 62	1086 ± 16	1562 ± 63	2.41	0.020
Cone counts								
Ellipsoids	**dorsal**	12.56 ± 0.50	12.33 ± 0.50	11.33 ± 0.61	13.22 ± 0.28	13.00 ± 0.62		
	medial	13.44 ± 0.97	14.56 ± 0.41	13.83 ± 0.60	15.11 ± 0.59	12.56 ± 0.50		
	ventral	11.56 ± 0.50	13.11 ± 0.45	13.83 ± 0.60	11.67 ± 0.50	12.78 ± 0.52	3.08	0.004
Nucleiis	**dorsal**	10.89 ± 0.56	10.33 ± 0.60	11.17 ± 0.60	11.56 ± 0.29	12.56 ± 0.87		
	medial	12.44 ± 0.53	14.00 ±0.41	12.83 ± 0.17	14.00 ± 0.59	12.33 ± 0.41		
	ventral	10.33 ± 0.47	12.11 ± 0.45	12.67 ± 0.49	11.67 ± 0.50	11.44 ± 0.60	2.82	0.007

PE = Pigment epithelium; OS = outer segments; EI = Ellipsoids; ONL = outer nuclei layer; OPL = outer plexiform layer; INL = inner nuclei layer; IPL = inner plexiform layer; GC = ganglion cells; WR = thickness of whole retina.